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<p>(54) Title: NEW POLYPEPTIDES, NUCLEIC ACID SEQUENCES ENCODING THEM, AND THEIR USE TO PREVENT THE ADHESION OF F107-FIMBRIATED BACTERIA (57) Abstract <p>The invention relates more particularly to a composition comprising at least one of the polypeptides, termed respectively FedE and FedF, in a substantially pure form, with said FedE and FedF having respective molecular weights of approximately 16 and 32 kDa as determined on Western blot, and with said FedE and FedF: each constituting approximately 1 % of the total protein content of F107 type fimbriae; and, each being nonessential to the normal assembly of F107 type fimbriae; and, both being essential for the adhesion of F107 type fimbriae to porcine small intestine; or a composition comprising fragments of FedE and/or FedF, or muteins thereof, insofar that said fragments and said muteins have retained the properties of the polypeptides as described above; and with said muteins consisting of substitutions and/or deletions and/or additions of one or several amino acids.</p></p>		

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NEW POLYPEPTIDES, NUCLEIC ACID SEQUENCES ENCODING THEM, AND THEIR USE TO PREVENT THE ADHESION OF F107-FIMBRIATED BACTERIA

The present invention relates to new polypeptides, nucleic acid sequences encoding them, and their use to prevent the adhesion of F107-fimbriated bacteria or bacteria carrying F107-related fimbriae to for instance the gastrointestinal tract of pigs.

Edema disease or *Escherichia coli* enterotoxemia in pigs is an *E. coli* infection mostly affecting just weaned animals. Typical signs of the disease are subcutaneous edema and neurological symptoms (Erskine et al., 1957; Soijka, 1965; Schimmelpfennig, 1970; Nielsen, 1986). Two virulence factors have been described in enterotoxemic *E. coli* strains, i.e. toxin Shiga-like toxin-II (SLT-IIv) and F107 fimbriae (for review see Imberechts et al., 1992a). Toxin SLT-IIv enters the circulation and induces characteristic lesions and symptoms (Erskine et al., 1957; Clugston et al., 1974; Clugston & Nielsen, 1974; Marques et al., 1987; MacLeod et al., 1991a; 1991b; 1991c). The properties of SLT-IIv and its role in the onset of clinical edema disease were profoundly studied (Clugston & Nielsen, 1974; Dobrescu, 1983; Smith et al., 1983; Gyles et al., 1988; Weinstein et al., 1988; MacLeod et al., 1991a; 1991b; 1991c). A second characteristic of enterotoxemic *E. coli* strains is their capacity to adhere to the small intestinal wall of infected pigs (Smith & Halls, 1968; Bertschinger & Pohlenz, 1983; Methiyapun et al., 1984). It has been suggested that colonization of the gut is a crucial step in the pathogenesis of the disease.

Postweaning diarrhea is a common *E. coli* infection of weaned pigs which may lead to serious diarrhea, dehydration and often death. Enterotoxigenic *E. coli* (ETEC) strains isolated from cases of postweaning diarrhea mainly belong to O groups O8, O141, O149 and O157 (Soijka, 1971; Wilson & Francis, 1986; Harel et al., 1991). These strains produce adhesion factors that allow colonisation of the gut, and enterotoxins that induce the intact intestinal mucosa to secrete fluid (Moon et al., 1979). In postweaning diarrhea strains fimbrial antigen F4 (K88), enterotoxins LTII (LT); STII (STb) and to a lesser extent STIa (STa), are the most often demonstrated virulence factors (Wilson & Francis, 1986; Harel et al., 1991). Colonisation factors such as F5 (K99), F6 (987P), and F41 may also be found, but are predominantly detected on neonatal enterotoxigenic *E. coli* isolates (Harel et al., 1991).

F107 fimbriae were demonstrated on the enterotoxemic *E. coli* strain 107/86 and were associated with the strain's ability to attach to isolated porcine brush border fragments (Bertschinger et al., 1990). Recently, the isolation and characterization of the F107 fimbriae and the cloning of a gene cluster containing the nucleotide sequence coding for the major subunit gene *fedA* was reported (Imberechts et al., 1992b). In this study, plasmids pIH2 and pIH120 were described which code for non-adhesive and adhesive F107 fimbriae, respectively. Moreover, it is apparent from this study that the presence of the major structural protein in the fimbrial structure is not in itself sufficient for adhesive capacity of the F107 fimbriae. By comparing the restriction maps of plasmids pIH2 and pIH120, it was suggested that the sequences responsible for the F107-specific adhesion could possibly be located downstream of *fedA* in the genetic determinant. This functional organization differs from that of K88 (F4), K99 (F5) and 987P (F6) fimbriae for which the adhesive property was localized on the major fimbrial proteins themselves (De Graaf & Klaasen, 1986; Jacobs et al., 1987a; Jacobs et al., 1987b).

E. coli contains a wide variety of antigenically different fimbriae, associated with various clinical symptoms and pathological lesions. The best known fimbrial systems include K88 and K99 fimbriae in enteric *E. coli*, and Pap fimbriae associated with urogenital tract *E. coli* infections (Gaastra & de Graaf, 1982). Certain homologies can be found among the *E. coli* fimbrial systems, based on e.g. amino acid sequences, receptor recognition, and organization of the fimbrial genetic determinant (Krogfelt, 1991). Nagy et al. (1992) demonstrated on enterotoxigenic strains of *E. coli* 2134P pili that morphologically resemble F107, and that even show cross-reaction with F107 fimbrial antigens. *E. coli* strains positive for 2134P adhered to small intestine of infected pigs, and induces diarrhea (Casey et al., 1992). Surface antigen "8813" was detected in more than eighty postweaning diarrhea strains that belonged to various serotypes (Salajka et al., 1992). The authors concluded from in vitro adhesion tests and experimental infection studies that "8813" was a new colonization factor. Finally, adhesive fimbriae were described on Australian postweaning strains of the O141 serotype (Kennan and Monckton, 1990). The subunit of isolated and purified fimbriae had a molecular mass of about 17 kDa. Therefore, it cannot be excluded that functional analogues of F107 fimbriae will be identified on yet other enteric, septicemic, urogenital or respiratory *E. coli* strains.

Fimbriae are polymers of a major protein subunit providing their structural backbone. In addition, some less abundant, minor fimbrial subunits may be integrated. The

genetic organization of *E. coli* type 1, Pap, S and F17 fimbriae has been defined (Normark et al., 1986; Lintermans, 1990; for review see: Hacker, 1990; Schmoll et al., 1990). Minor proteins PapE, PapF and PapG which are encoded by genes located downstream from the major subunit gene *papA* are involved in the digalactoside-specific binding of Pap fimbriae (Lindberg et al., 1984). Fimbriated bacteria or isolated fimbriae from *papF* or *papG* mutants have lost their adhesive activity (Lindberg et al., 1986). Protein PapG was identified as the receptor binding protein (Uhlén et al., 1985; Lindberg et al., 1986; Lindberg et al., 1987; Lund et al., 1987). Protein PapE is the structural protein of the fibrillar filament at the tip of the Pap fimbriae, and acts as a carrier for adhesin PapG (Lindberg et al., 1986; Lund et al., 1987; Kuehn et al., 1992). The length of Pap fimbriae is determined by the proteins PapF and PapH. PapF is thought to initiate the fimbrial biosynthesis (Lindberg et al., 1986; Lindberg et al., 1987; Lund et al., 1987). Protein PapH is inserted in the assembled fimbrial structure and prevents the further polymerization of structural proteins (Båga et al., 1987).

Similarly, genes *fimF*, *fimG* and *fimH* were identified downstream from *fimA* at the distal end of the genetic determinant of type 1 fimbriae (Klemm, 1982; Klemm et al., 1985). Protein FimH was characterized as the type 1 fimbrial adhesin, since *fimH* mutants were unable to adhere to guinea pig erythrocytes (Klemm and Christiansen, 1987). In contrast, *fimF* and *fimG* mutants were still adhesive. All three gene products FimF, FimG and FimH were shown to play a role in determining the length of type 1 fimbriae (Klemm et Christiansen, 1987). Also in the gene cluster coding for S fimbriae, the adhesin gene *sfaS* and two genes *sfaG* and *sfaH* coding for minor subunits were identified downstream from the structural gene (Schmoll et al., 1989; Schmoll et al., 1990). Proteins SfaS and SfaH seem to be involved in the modulation of fimbriation (Schmoll et al., 1989). Finally, the F17 fimbrial adhesin was identified, and its gene *F17-G* was localized at the distal end of the gene cluster (Lintermans et al., 1991). No other minor subunit genes were identified in the F17 gene cluster (Lintermans, 1990).

The present invention aims at providing compositions including proteins being present in minor amounts in F107 type fimbriae and which are implicated in the adhesive functions of these type of fimbriae.

The present invention aims at providing the complete nucleotide sequence of the genes *fedE* and *fedF* implicated in the adhesive functions of F107 type fimbriae, for which the genes are located downstream from the *fedA* gene.

The present invention also aims at designing vaccine compositions for immunizing against the F107 type adhesins.

It is another object of the present invention to provide therapeutic compositions which can be used to treat edema disease, postweaning diarrhea, or any other disease state caused by the adhesion of F107 type fimbriated bacteria.

Moreover, the present invention aims at providing a vaccine for preventing the attachment of F107 type fimbriated bacteria to the specific cell types to which they adhere *in vivo*, preferably the digestive tract of young pigs.

The present invention also aims at using F107 type fimbriae-derived presentation vectors for inducing an immune response to any antigen of choice.

The present invention also aims at designing a drug screening process for identifying drugs which interfere with the adhesion of F107 type fimbriated bacteria to the specific cell types to which they adhere *in vivo*, preferably the digestive tract of young pigs.

The invention relates more particularly to a composition comprising at least one of the polypeptides, termed respectively FedE and FedF, in a substantially pure form, with said FedE and FedF having a respective molecular mass of approximately 16 and 32 kDa as determined on Western blot, and with said FedE and FedF:

- * each being present in F107 type fimbriae in a minor amount which constitutes approximately 1 % of the total protein content of these fimbriae; and,

- * each being nonessential to the normal assembly of F107 type; and,

- * both being essential for the adhesion of F107 type fimbriae to porcine small intestine;

or a composition comprising fragments of FedE and/or FedF, or muteins thereof, insofar that said fragments and said muteins have retained the properties of the polypeptides as described above; and with said muteins consisting of substitutions and/or deletions and/or additions of one or several amino acids.

The expression "F107 type fimbriae" relates to fimbriae such as F107 fimbriae, 2134P pili, surface antigen 8813, fimbriae from Australian postweaning diarrhea strains of the 0141 serotype, all as reviewed above, or any other fimbriae characterized by a major structural component FedA having an amino acid sequence homology of at least 75%, preferably at least 85%; more preferably at least 95% to any of the amino acid sequences as depicted in SEQ ID NO 6 to 21; or a nucleic acid sequence homology of at

least 70%, preferably at least 80%, more preferably at least 91% to any of the nucleic acid sequences depicted in any of SEQ ID NO 6, 8, 10, 12, 14, 16, 18, or 20.

The expression "substantially pure form" means a purity grade of at least 95% and more particularly of at least 98%, and more preferably of 100%.

The expression "constituting approximately 1% of the total protein content of F107 type fimbriae" refers to the fact that both FedE and FedF are present in a minor amount in F107 fimbriae as determined by Western blot experiments explained in detail in the example section.

The expression "each being nonessential for normal assembly" refers to the fact that even if both FedE and FedF are absent and *a fortiori* either FedE or FedF is present, normal assembly of F107 type fimbriae occurs. The normal assembly of F107 type fimbriae refers itself to structural characteristics of the F107 type fimbriae as revealed by means of electron microscopy after negative staining as demonstrated in the example section. Indeed, electron microscopic examination revealed the presence of fimbriae on the bacterial surface of *E. coli* transformants HB101 (pIH121) and HB101(pIH122) (see example section) containing an insertion mutation in gene *fedE*, as well as on *E. coli* transformants HB101 (pIH126), HB101(pIH128) and HB101(pIH129); (see example section) with an insertion inactivation of gene *fedF*. Therefore, gene products FedE and FedF are not essential for the production or assembly of F107 fimbriae.

The expression "both being essential for the adhesion of F107 type fimbriae" refers to the fact that no adhesion of mutant F107-fimbriated bacteria occurs when either FedE or FedF is absent from the "mutant" F107 fimbriae. The adhesiveness of bacteria can be studied in a variety of ways, a few of which are presented below and documented further in the example section of this application. These tests are also valuable for studying the impact of FedE/FedF-specific antibodies or isolated FedE/FedF on the adhesive properties of fimbriated bacteria.

The following types of *in vitro* tests can be used to determine the presence or absence of adhesion. These tests for adhesion are not limited to the gastrointestinal tract but can be used to test adhesion of F107 type fimbriated bacteria to any type of tissue.

Qualitative *in vitro* adhesion test using isolated porcine villi. Villi from just weaned susceptible pigs were scraped from the mucosa of the lower part of the small intestine. The scrapings were collected in Krebs medium, supplemented with 1% formol (Krebs medium consists of 120 mM NaCl, 14 mM KCl, 25 mM NaHCO₃ and 1 mM

KH_2PO_4). This sampling must be done as soon as possible after the death of the animal to circumvent possible postmortem changes. After several washes with Krebs buffer, the villi may be aliquoted and stored at -80°C in a medium, composed of 80 ml Hanks modified medium containing 0.5% lactalbumin, 10 ml fetal calf serum [GIBCO-BRL], 30 ml DMSO [Merck] and 30 ml glycerine [Merck]. Hanks medium consists of 1.37 M NaCl, 54 mM KCl, 8 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3 mM $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 4 mM KH_2PO_4 , 10 mM CaCl_2 and 1% glucose. For use, villi aliquots were thawed and were washed once with Krebs supplemented with 1% formol, and three times with Krebs. The test was done by incubating villi for half an hour at room temperature with a suspension of the bacteria under study. Attachment was ascertained by means of phase contrast microscopy.

Brush border membrane test. Whereas the above-mentioned *in vitro* adhesion test is mainly qualitative, the attachment of bacteria may also be quantified by examining their binding to purified brush border membranes which are coated on microtiter plates (Hendriks et al., 1987). The brush border membrane technique may be valuable to study possible differences in affinity between HB101(pIH120) and *fedE* and *fedF* mutants. Moreover, attachment inhibition studies with polyclonal anti-FedE or anti-FedF antibodies or preferably monoclonal anti-FedE or anti-FedF antibodies may be useful to identify the F107 type receptor binding protein.

Adsorption to isolated brush border membranes was described earlier to quantitate the attachment of bacteria to intestinal cells (Hendriks et al., 1987).

Hemagglutination. Agglutination of red blood cells is an easy and quantitative test that can be used to demonstrate attachment properties of bacteria. However, *E. coli* strain 107/86 is not able to agglutinate erythrocytes from gibbon, horse, cattle, sheep, pig, dog, cat, guinea pig and chicken (Bertschinger et al., 1990). However, red blood cells from other species still may be checked for possible agglutination.

Eukaryotic cell lines. The adhesive capacity of *E. coli* strain 107/86 or other F107 type fimbriae bearing bacteria to eukaryotic cell lines has not been investigated yet. Nevertheless, it may be a valuable method to circumvent the variability of villi and brush border preparations. Preferably, cultured Caco-2 (adenocarcinoma from a human colon), HT29 and T84 cell lines should be used, because upon culturing they are polarized, thus maximally resembling intestinal cells. Other eukaryotic cells may include Vero (African green monkey kidney cells), HEP-2 (carcinoma of a human larynx), HeLa (human cervix) cells, and MDCK (Madine Darby Canine kidney).

Laboratory animal model. Various lines of mice, guinea pigs, hamsters or rabbits may be colonized by *E. coli* strain 107/86 or other F107 type fimbriae carrying bacteria (*E. coli* 107/86 related strains), and thus serve as a model for edema disease in pigs. In these tests, 10^4 to 10^5 *E. coli* 107/86 (or related strains) carrying the gene for streptomycin resistance are given orally to the animal, and the excretion of test bacteria with the feces is examined daily. According to this model system, adhesion results in a prolonged retention time. Due to its streptomycin resistance (400 $\mu\text{g/ml}$), the test bacteria are easily determined from other colonizing bacteria by plating diluted suspensions of feces expected to contain *E. coli* strain 107/86 (or the related strain) on selective media. The excretion is indicated as the number of colony forming units per gram feces. Strain 107/86 (or related strains) may also be identified by its expression of F107 type fimbriae (immune detection on colony blot) and by the presence of F107 type genes (dot-blot hybridization or polymerase chain reaction). Alternatively, the animals may be sacrificed at a defined moment after oral infection, and colonization of the intestine can be investigated by incubation of mucosal scrapings or homogenized segments of the intestine on bacterial growth medium and histologic, immunofluorescence or electron microscopic examination of preparations of the intestine.

Infection of just weaned pigs. Colonization of the natural host by *E. coli* strain 107/86 was reported (Bertschinger et al., 1990) and can be combined with any of the above-mentioned methods.

Table A gives an overview of the amino acid substitutions which could be the basis of some of the muteins as defined above.

TABLE A

Amino acids	Synonymous groups
Ser	Ser, Thr, Gly, Asn
Arg	Arg, His, Lys, Glu, Gln
Leu	Leu, Ile, Met, Phe, Val, Tyr
Pro	Pro, Ala, Thr, Gly
Thr	Thr, Pro, Ser, Ala, Gly, His, Gln
Ala	Ala, Pro, Gly, Thr
Val	Val, Met, Ile, Tyr, Phe, Leu, Val
Gly	Gly, Ala, Thr, Pro, Ser
Ile	Ile, Met, Leu, Phe, Val, Ile, Tyr

Phe	Phe, Met, Tyr, Ile, Leu, Trp, Val
Tyr	Tyr, Phe, Trp, Met, Ile, Val, Leu
Cys	Cys, Ser, Thr
His	His, Gln, Arg, Lys, Glu, Thr
Gln	Gln, Glu, His, Lys, Asn, Thr, Arg
Asn	Asn, Asp, Ser, Gln
Lys	Lys, Arg, Glu, Gln, His
Asp	Asp, Asn, Glu
Glu	Glu, Gln, Asp, Lys, Asn, His, Arg
Met	Met, Ile, Leu, Phe, Val

The peptide fragments according to this preferred embodiment of the invention can be prepared by classical chemical synthesis.

The synthesis can be carried out in homogeneous solution or in solid phase.

For instance, the synthesis technique in homogeneous solution which can be used is the one described by Houbenweyl in the book entitled "Methoden der organischen chemie" (Method of organic chemistry) edited by E. Wunsh, vol. 15-I et II. THIEME, Stuttgart 1974.

The polypeptides of the invention can also be prepared in solid phase according to the methods described by Atherton and Shepard in their book entitled "Solid phase peptide synthesis" (IRL Press, Oxford, 1989).

The presence of F107 type fimbriated *E. coli* bacteria is not restricted to the gastrointestinal tract of pigs. They can also colonize the respiratory and/or urogenital tract of other species, including cattle, sheep, goats, horses, cats, dogs, and man. For example, it is known that K99 fimbriae can be identified on *E. coli* isolates from pigs as well as ruminants, including calves and lambs (Gaastra & de Graaf, 1982). F41 fimbriae were found in association with both calves and piglets (Morris et al., 1982; Morris et al., 1983). The major structural subunit of F17 fimbriae, prepared from *E. coli* strains isolated from newborn calves with diarrhea, shows homology to that of G fimbriae found in association with human pyelonephritogenic *E. coli* strains (Lintermans, 1990). Therefore, it cannot be excluded that one type of fimbriae (such as F107) with one particular tropism can evolve or may have evolved to an antigenically related type of fimbriae with a new tropism.

Consequently the present invention also relates to proteins which are both

structurally and functionally related to the FedE and/or FedF proteins of F107 type fimbriated bacteria as illustrated in the example section. These proteins are generally referred to in this work as FedE and/or FedF polypeptides of F107 type fimbriae. Specific examples of F107 type fimbriae include 2134P pili present on enterotoxigenic strains as described by Nagy et al. (1992), "8813" antigen present in postweaning diarrhea strains belonging to different serotypes as described by Salajka et al. (1992) and adhesive fimbriae described for Australian postweaning strains of the 0141 serotype (Kennan and Monckton, 1990). The complete sequence of the *FedA* gene structural component of these F107 type fimbriae is disclosed in the examples section of the present invention. Using primers derived from this sequence, the complete sequence of the FedE/FedF genes of these F107 type fimbriae may be determined. It is, however, to be understood that also other F107 type fimbriae bearing strains may be identified in the future. These FedE/FedF proteins should show at least 50 to 60% homology, preferably more than 70 to 80%, more preferably more than 90 to 95%, and most preferably more than 98 to 99% homology on the amino acid sequence level with FedE and FedF as disclosed in SEQ ID NO 1 or 2. Moreover, said FedE/FedF are present in minor amounts in the F107 type fimbriae; are nonessential for the normal assembly of the F107 type fimbriae; and, are essential for adhesion to the cell type to which they adhere *in vivo* as assayed by means of *in vitro* adhesion tests as described above. Moreover these FedE and/or FedF proteins may recognize a receptor molecule present on the surface of the cell type to which they adhere *in vivo*, analogous to the receptor molecule recognized by the F107 FedE and/or FedF proteins as disclosed in SEQ ID NO 1 and 2. In this respect, a monoclonal antibody raised against at least FedE or FedF which has been selected for its property to inhibit the adhesion of F107 type fimbriae to the small intestine microvilli, may also interfere with the binding of the other F107 type *E. coli* bacteria to the cell type to which they adhere *in vivo*.

According to a preferred embodiment of the present invention, the above-defined compositions comprise at least one of the polypeptides FedE or FedF, with said FedE and FedF being characterized by their respective amino acid sequences as shown in SEQ ID NO 1 and 2.

The total amino acid sequences of FedE and FedF are also shown in Figure 6.

The polypeptide FedE of SEQ ID NO 1 is new, and is part of the invention.

The polypeptide FedF of SEQ ID NO 2 is new, and is also part of the invention.

The present invention also relates to a polynucleic acid in substantially isolated form constituted by, or comprising a contiguous sequence of at least 10 nucleotides selected from:

(a) the polynucleic acids which code for any of the FedE or FedF polypeptides as defined above,

(b) the polynucleic acid sequences which hybridize to any of the polynucleic acids as defined in (a), or,

(c) the polynucleic acid sequences which are degenerate as a result of the genetic code to the polynucleic acid sequences as defined in (a) or (b) and which either code for a polypeptide as defined above, or hybridize to a polynucleic acid which codes for a polypeptide as defined above.

The term "hybridizes to" refers to conventional hybridization conditions known to the man skilled in the art, preferably to stringent hybridization conditions.

The polynucleic acids of the invention are to be understood as also comprising the degenerate nucleic acid sequence of the nucleic acids coding for the above-mentioned polypeptides of the invention.

The term "polynucleic acids" corresponds to either double-stranded or single-stranded cDNA or genomic DNA, or RNA; and possibly also to these nucleic acids chemically modified according to any technique known in the art.

The polynucleic acids according to this embodiment of the present invention can be determined by means of a process comprising the steps of:

- preparing mRNA from bacterial cells (step 1),
- performing an amplification reaction, such as PCR, of mRNA, with primers essentially consisting of, or comprising a nucleotide sequence containing at least part of a nucleotide sequence encoding a FedA major structural fimbrial component as defined above, preferably from the nucleotide sequences of *fedA* genes as represented in SEQ ID NO 6 to 21, to obtain the amplified products (step 2).

The present invention relates more particularly to the nucleic acid shown in SEQ ID NO 3, and more preferably those containing the FedE and/or FedF coding regions as shown in SEQ ID NO 4 and 5.

SEQ ID NO 3 corresponds to the nucleic acid defined by nucleotide at position 323 to nucleotide at position 2815, on figure 6.

SEQ ID NO 4 corresponds to the nucleic acid defined by nucleotide at position 592 to nucleotide at position 1104, on figure 6.

SEQ ID NO 5 corresponds to the nucleic acid defined by nucleotide at position 1405 to nucleotide at position 2304, on figure 6.

The total nucleic acid sequence of the nucleic acid containing both genes *FedE* and *FedF*, as well as their coding regions, is shown in Figure 6.

The present invention relates also to a probe containing at least 10 nucleotides, preferably containing from about 15 to about 50 nucleotides, with said probe being able to detect the presence of a polynucleic acid as described above or the complementary sequence thereof, in a biological sample, by hybridizing to a target region present in a polynucleic acid sequence as defined above, more particularly to a polynucleic acid sequence contained in any of SEQ ID NO 3, or SEQ ID NO 4, or SEQ ID NO 5; and with said probe being possibly chemically modified.

The term "probe" refers to a single stranded sequence-specific oligonucleotide having a sequence which is complementary to a target sequence, present in F107 type *fedE/fedF* gene cluster, to be detected or cloned. Probes may be labelled according to any of the techniques known in the art. Preferably these probes are about 15 to 50 nucleotides long. Such a probe may be composed of DNA, RNA, or synthetic nucleotide analogs. The probes of the invention can be incubated with an analyte strand immobilized to a solid substrate. In a preferred embodiment of the invention, the probes themselves can be immobilized to a solid substrate. These probes may further include capture probes, characterized as being coupled to a binding molecule which in turn is directly or indirectly bound to a solid substrate, or may also include label probes, characterized in that they carry a detectable label.

The expression "biological sample" may refer to any biological sample (tissue or fluid) containing F107 type *fedE/fedF* fimbrial cluster sequences and refers more particularly to the content of feces, eventually washes from the respiratory tract, or the content from urogenital tract, or organs including lymph nodes, liver and spleen. The detection of hybrids formed between the target region, if present, and the probes as mentioned above depends on the nature of the reporter molecule used (either present on the probe or on the analyte strand to be targeted) and may be determined by means of colorimetric, fluorescent, radiometric detection or any other method comprised in the state of the art.

The present invention relates also to a recombinant vector, particularly for cloning and/or expression, with said recombinant vector comprising a vector sequence, and at least a part of any of the polynucleic acid sequences as defined above, more particularly the nucleic acid sequences shown in SEQ ID NO 3, SEQ ID NO 4, or SEQ ID NO 5; and further characterized in that the coding sequence is operably linked to control sequences capable of providing for the expression of the coding sequence by the specific host cell.

The term "operably linked" refers to a juxtaposition wherein the components are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence.

The term "control sequence" refers to those sequences which control the transcription and/or translation of the coding sequences; these may include but are not limited to promoter sequences, transcriptional initiation and termination sequences, and translational initiation and termination sequences. In addition, control sequences refer to sequences which control the processing of the polypeptide encoded within the coding sequence; these may include, but are not limited to sequences controlling secretion, protease cleavage, and glycosylation of the polypeptide.

The term "recombinant vector" may include a plasmid, or a phage, a cosmid or a virus.

The present invention relates also to an appropriate bacterial host transformed with a recombinant vector as defined above.

According to a preferred embodiment, the present invention also relates to a recombinant polypeptide expressed by culturing a suitable bacterial host as defined above, which is transformed with a recombinant vector as defined above, with said recombinant polypeptide being possibly part of a fusion protein, consisting of any nonhomologous protein, chosen to optimize the expression of the polypeptide or to bring about any desired side-effect to the resulting fusion product.

In order to carry out the expression of the polypeptides of the invention in bacteria, such as *E. coli*, the following steps are required:

- transformation of an appropriate bacterial host with a recombinant vector in which a nucleotide sequence coding for one of the polypeptides of the invention has been inserted (insert) under the control of the appropriate regulatory elements, particularly a promoter recognized by the polymerases of the bacterial host and, an appropriate ribosome binding site (RBS), enabling the expression in said bacterial host of said

nucleotide sequence,

- culture of said transformed bacterial host in conditions enabling the expression of said insert

- possibly, purification of the recombinant polypeptide by any of the techniques known in the art.

The present invention thus also relates to a method for producing a recombinant polypeptide as defined above, comprising incubating a host cell as defined above under conditions that provide the expression of the coding sequences.

The present invention also relates to an antibody characterized as being specifically directed against any of the polypeptides, muteins thereof, or fragments thereof, as defined above, with said antibodies being preferably monoclonal antibodies; or an antigen-binding fragment thereof, of the $F(ab')_2$, $F(ab)$ or single chain F_v type, or any type of recombinant antibody derived thereof.

Antibodies according to this preferred embodiment of the invention include specific polyclonal antisera prepared against FedE and/or FedF, or fragments thereof, or muteins thereof, and having no cross-reactivity to others proteins, or monoclonal antibodies prepared against FedE and/or FedF, or fragments thereof, or muteins thereof.

The monoclonal antibodies of the invention can be produced by any hybridoma liable to be formed according to classical methods from splenic cells of an animal, particularly of a mouse or rat, immunized against the adhesive polypeptides according to the invention, or muteins thereof, or fragments thereof, defined above on the one hand, and of cells of a myeloma cell line on the other hand, and to be selected by the ability of the hybridoma to produce the monoclonal antibodies recognizing the polypeptides which have been initially used for the immunization of the animals.

The monoclonal antibodies according to this preferred embodiment of the invention may be humanized versions of the mouse monoclonal antibodies made by means of recombinant DNA technology, departing from the mouse and/or human genomic DNA sequences coding for H and L chains or from cDNA clones coding for H and L chains.

Also fragments derived from these monoclonal antibodies such as Fab, $F(ab)_2$ and ssFv ("single chain variable fragment"), providing they have retained the original binding properties, form part of the present invention. Such fragments are commonly generated by, for instance, enzymatic digestion of the antibodies with papain, pepsin, or other proteases. It is well known to the person skilled in the art that monoclonal

antibodies, or fragments thereof, can be modified for various uses.

The antibodies involved in the invention can be labelled by an appropriate label of the enzymatic, fluorescent, or radioactive type.

The invention also relates to the use of the proteins of the invention, muteins thereof, or fragments thereof, for the selection of recombinant antibodies by the process of repertoire cloning (Perrson et al., 1991).

According to a preferred embodiment of the present invention, an antibody, or an antigen-binding fragment $F(ab')_2$, $F(ab)$, single chain F_v and all types of recombinant antibodies, as defined above are further characterized in that they can inhibit the adhesion of F107 type *E. coli* strains to the specific cell type to which they attach *in vivo*, as assayed in any of the *in vitro* systems as described above, and for instance characterized in that they inhibit the adhesion of F107 *E. coli* strains to the porcine intestinal wall by at least 10%, preferably by at least 25%, and more preferably by more than 50%. The *in vitro* adhesion assay can be used to test either isolated F107 type fimbriae (including fimbrial components or molecules, and compounds containing fragments, or muteins of these fimbrial components), or antibodies (raised against F107 type fimbriae or its derived components, or muteins or fragments thereof) to inhibit the adhesive capacity of F107 type bearing bacteria. To this end, isolated porcine intestinal villi, brush border membranes, red blood cells, eukaryotic cell lines or isolated receptors present on the cell type to which these fimbriated bacteria adhere, preferentially the villi of the porcine intestine wall are incubated with the preparation of F107 type fimbriae, or the components therefrom as described above in the presence or absence of interfering substances. Depending on the detection system of the assay (visual, spectrophotometric, or agglutination titer), adhesion or interference with adhesion of F107 type fimbriated bacteria can be determined.

According to another embodiment, the present invention also relates to a method for detecting the presence of F107 type fimbriated bacteria, in a sample liable to contain these bacteria, comprising the steps of:

- contacting the sample to be analyzed with appropriately labeled antibodies as defined above;
- detecting the presence of the labeled F107 type fimbrial antigen-antibody complex formed.

The term "sample" may refer to any fluid or tissue sample derived from any origin liable to contain F107 type fimbriated bacteria, preferably intestinal content of feces,

eventually washes from the respiratory tract, or content from the urogenital tract, or organs, including lymph nodes, liver and spleen.

The term "label" may refer to any detectable label, chosen from the group of enzymatic, fluorescent, or radioactive labels comprised in the art.

It is to be understood that departing from the amino acid sequence of the FedE or FedF proteins, monoclonal antibodies may be selected which react uniquely with the fimbriae of a certain type of F107 type fimbriated bacteria. Such monoclonal antibodies may form the basis of a typing procedure to differentiate the epidemiological spreading of F107 type bacteria or to distinguish which type(s) of F107-related bacteria is (are) present in a biological sample to be analyzed:

According to an alternative embodiment, the invention relates to a process for detecting the presence of bacteria containing F107 type fimbrial *fedE/fedF* gene clusters, in a sample liable to contain these bacteria, comprising the steps of :

- possibly performing a PCR amplification step with suitable primers,
- contacting either the nucleic acids of the sample, appropriately released, or the amplified PCR product, with a probe as defined above, liable to target either the nucleic acid sequences as shown in SEQ ID NO 3 to 5, or a nucleic acid sequence showing 50 to 60% homology, preferably more than 70 to 80%, more preferably more than 90 to 95%, and most preferably more than 98 to 99% homology to the sequences shown in SEQ ID NO 3 to 5.

This embodiment of the invention is based on the detection of a polynucleic acid encoding any of the genes *fedE* and/or *fedF* (as represented in SEQ ID NO 3 to 5) or homologous genes, present in a biological sample, liable to contain these *E. coli* strains. Detection can be done on the sample with or without prior amplification of the nucleic acids present in the sample. Amplification of the analyte strand present in the biological sample may be done by means of polymerase chain reaction (PCR) using suitable primers (f.i. *fedA* gene primers as described above or *fedE/fedF* primers if available); with said amplification being repeated between 20 and 80 times. Nucleic acids are detected by means of either labelling the probes used for detection or by incorporating a label during the amplification step by means of methods known in the art.

According to a preferred embodiment of the invention, the analyte strand may be enzymatically or chemically modified either *in vivo* or *in vitro* prior to hybridization. Many systems for coupling reporter groups to nucleic acid compounds have

been described, based on the use of such labels as biotin and digoxigenin. In still another embodiment of the invention a sandwich hybridization may be used. In a preferred embodiment, the target sequence present in the analyte strand is converted into cDNA, with said cDNA being amplified by any technique known in the art such as the polymerase chain reaction (PCR; Saiki et al., 1988), ligase chain reaction (LCR; Landegren et al. 1988; Wu & Wallace, 1989; Barany, 1991), nucleic acid sequence-based amplification (NASBA; Guatelli et al., 1990; Compton, 1991), transcription-based amplification system (TAS; Kwoh et al., 1989) strand displacement amplification (SDA; Duck et al., 1990, Walker et al., 1992) or amplification by means of Q β replicase (Lizardi et al., 1988, Lomeli et al., 1989).

The cDNA amplification step is preferably achieved by means of PCR technology and may consist of the following steps :

(a) providing a set of primers for a polymerase chain reaction method which flank the target sequence to be detected (*fedA* and/or *fedE/fedF* primers);

(b) amplifying the target region via a polymerase chain reaction method by means of the primers of (a); and in the same step an appropriate label molecule can be incorporated into the amplified target sequence, with said label molecule being preferably digoxigenin or biotin.

The term "primers" corresponds to oligonucleotide sequences being complementary to conserved regions of sense or antisense strands of cDNA or RNA derived from the fimbrial *fedA* and/or *fedE/fedF* gene clusters.

The presence (existence) of F107 type fimbriae in a biological sample is commonly concluded on the basis of the comparison between formation of F107 fimbrial antigens in the intestinal content of infected pigs, and the presence of fimbriae, as detected by immunological techniques as indicated above, or by the demonstration of the presence of the *fed A* gene in the isolated *E. coli* strain. Preferably, F107 type positive bacteria should be identified on the basis of the presence of FedE and/or FedF proteins or their gene products. Some edema disease associated *E. coli* strains produce F107 antigens in the intestine, as demonstrated with immunofluorescence on mucosal smears, although the *fedA* gene was not demonstrated by means of PCR (Imberechts et al., unpublished data). It is possible that these isolates have a colonization factor genetically different from, but antigenically similar to F107 fimbriae. Consistent with this observation, it seems more appropriate to diagnose F107-positive bacteria in the sample by demonstrating the presence

of the *fedE* and/or *fedF* gene products. Moreover, since both gene products FedE and FedF are needed for colonization of the epithelial surface, they represent the fimbrial virulence factor and are as such important to include in the diagnosis.

It is to be understood that departing from the nucleic acid sequence of the FedE or FedF proteins or the related genes, specific probes may be selected which react uniquely with the fimbriae of a certain type of F107-related bacteria. Such specific probes may form the basis of a typing procedure to differentiate the epidemiological spreading of F107 type bacteria or to distinguish which type of F107 type bacteria are present in a biological sample to be analyzed.

According to another embodiment, the present invention also relates to antisense peptides prepared against FedE and/or FedF, or fragments thereof, or muteins thereof, as defined-above.

More particularly, the term "antisense peptide" is reviewed by Blalock (1990) and by Roubos (1990). In this respect, the molecular recognition theory (Blalock, 1990) states that not only the complementary nucleic acid sequences interact but that, in addition, interacting sites in proteins are composed of complementary amino acid sequences (sense ligand with receptor or sense ligand with antisense peptides). Thus, two peptides derived from complementary nucleic acid sequences in the same reading frame will show a total interchange of their hydrophobic and hydrophilic amino acids when the amino terminus of one is aligned with the carboxy terminus of the other. This inverted hydropathic pattern might allow two such peptides to assume complementary conformations responsible for specific interaction.

The antisense peptides can be prepared as described in Ghiso et al. (1990). By means of this technology it is possible to logically construct a peptide, having a physiologically relevant interaction with a known peptide, by simple nucleotide sequence analysis for complementarity, and synthesize the peptide complementary to the binding site.

According to another embodiment, the present invention relates to a pharmaceutical composition containing or consisting of at least one of the polypeptides as defined above, or fragments thereof, or muteins thereof, or any of the antibodies, or any of the antisense peptides as defined above.

The aim of the therapeutic administration of compositions including as active substances any of the polypeptides FedE and/or FedF, or fragments thereof, or muteins

thereof or antibodies derived thereof, or antisense peptides derived thereof, as described above is that they would inhibit *in vivo* the binding of F107 type bacteria, to the specific cell type to which they attach, preferentially the porcine small intestine villi as for F107-fimbriated bacteria. The pharmaceutical compositions according to the invention can be administered orally, intranasally, or via any other route known in the art.

The aim of the passive protection of pigs with F107-FedE and/or FedF antibodies, is to supply antibodies orally to pigs at weaning in order to provide them with a local protection at the moment that the animals are most susceptible for the disease. Therefore, chickens are immunized (Lüscher et al., 1986) with F107 type fimbriae, and their anti-F107-immunoglobulin-containing eggs are used either lyophilized, or as total eggs (without prior purification of the antibodies) in protection studies (Schmidt et al., 1986), on the condition that the antibodies display the property of anti-adhesive capacity in an *in vitro* adhesion test. The egg antibodies are partially capable of resisting digestive activities (acid and proteolysis) in the gastrointestinal tract (Lüscher et al., 1986; Schmidt et al., 1986). For the passive protection of pigs a continuous uptake of lyophilized eggs with the feed is needed, starting at the moment immediately after weaning in order to prevent the colonization by an incoming pathogen. The duration and dosage of this feed supplement is determined according to techniques comprised in the art. Similar passive protection studies may be developed with eggs from chickens immunized with SLT-IIv toxoid.

Pharmaceutical compositions based on isolated and purified F107 type fimbriae or antigens thereof aim at the inhibition of binding of F107 type fimbriated bacteria to the intestinal mucosa of pigs. To investigate if isolated F107 fimbriae can bind to porcine villi, an indirect immunofluorescence test was set up. Isolated crude fimbrial extracts, prepared by thermoelution (20 minutes at 60°C) and by mechanical shearing (passing of the bacterial culture 10 times through a G-25 needle) of a 1-ml bacterial suspension containing $\pm 10^9$ bacteria of strains 107/86 and HB101(pIH120) were incubated with the villi, and subsequently with anti-F107-antiserum, diluted 1/100. The conjugate consisted of goat anti-rabbit IgG FITC, at a dilution of 1/100 in Evans blue 1/10000. As negative control, a fimbrial extract of strain HB101(pUC18) was used. In the samples containing villi incubated with fimbriae from *E. coli* strains 107/86 and HB101(pIH120) fluorescence was detected along the epithelial surface of the villi. Also mucus protruding from goblet cells was stained. No such image was seen in the negative control. These results indicate that, irrespective of the method of fimbriae preparation, the adhesive

capacity of the fimbriae is not lost during the extraction. In a second experiment fimbrial extracts were prepared by heat treatment from transformants containing *fedE* (pIH121, pIH122) and *fedF* (pIH126, pIH128, pIH129) mutants. All samples were negative, or showed only a limited fluorescence dye linked to mucus. An exception was the fimbrial preparation of HB101(pIH129) in which a staining was observed of the epithelial surface comparable to that obtained with fimbriae prepared from HB101(pIH120). The immunofluorescence shown in the various fimbrial extracts therefore completely confirms the *in vitro* adhesion test results with their corresponding transformants.

The invention also relates to the use of any of the polypeptides, fragments thereof, or muteins thereof, or antibodies, or antisense peptides, as defined above, for the preparation of a medicament for treating edema disease, postweaning diarrhea, or any other disease states caused by the adhesion of F107 type fimbriated bacteria.

Examples of other possible infections which can be treated with the medicament according to this embodiment include urogenital tract infections as well as respiratory tract infections which may be caused by F107 type fimbriae carrying *E. coli* strains.

The present invention also relates to a vaccine composition containing or consisting of at least one of the polypeptides, or fragments thereof, or muteins thereof as defined above, possibly in combination with polypeptide B of the toxin of SLT-IIv, or the toxoid, with said polypeptides or fragments thereof, or muteins thereof, being able to raise antibodies capable of blocking the adhesion of F107 type fimbriated bacteria to the cells to which they attach assayed as defined above, or of raising toxin-inactivating (or neutralizing) antibodies.

The active ingredients of the vaccine composition may be administered orally, intranasally, or via any other route known in the art.

In this respect, it was unambiguously demonstrated that edema disease strains of *E. coli* produce a toxic substance that is responsible for the characteristic lesions and symptoms of the disease (MacLeod et al., 1991b). Lysates of edema disease strains of *E. coli* possess Vero cell cytotoxicity and, therefore, this property is used as an indicator of SLT-IIv toxin. Based on amino acid sequence homology, SLT-IIv resembles Shiga toxin which is composed of one A subunit and 5 B subunit copies. The A part of Shiga toxin and SLT-IIv have RNA glycosidase activity which specifically removes a particular adenine residue in the 28S subunit of eukaryotic rRNA (Saxena et al., 1989). In this way, the

cellular protein synthesis is inactivated and the metabolic functions of the cell are disorganized. The B subunit recognizes globotetraosylceramide and thus bears the receptor binding part of the toxin (De Grandis et al., 1989; Samuel et al., 1990). It was demonstrated that SLT-IIv inhibited the protein synthesis of porcine aortic endothelial cells, proving that at least some endothelial tissue possesses the toxin-specific receptor (MacLeod et al., 1991a). Thus, characteristic lesions and symptoms may be explained by the vasotoxic activity of the SLT-IIv toxin.

Until now, only vaccination studies with SLT-IIv toxin were described. No vaccination with isolated and/or purified F107 fimbriae, or other adhesion factors, or combinations of fimbriae, other adhesion factors and toxin components have been reported to protect pigs against edema disease.

Successful subcutaneous vaccination was reported in pigs, one week before weaning, with a product that consisted of a crude toxin preparation that was inactivated with glutaraldehyde (Awad-Masalmeh et al., 1989). Morbidity, mortality and excretion of the pathogenic bacteria after challenge were significantly decreased in comparison with the control group. Similar experiments were done by injecting 2- and 6-weeks-old pigs intramuscularly with 25 μ g of a highly purified, inactivated SLT-IIv preparation (MacLeod and Gyles, 1991). This vaccination was repeated after two weeks, and one week later the pigs were challenged by intravenous injection of non-detoxified SLT-IIv toxin. All the pigs raised neutralizing antibodies against the toxin and survived the challenge. However, the animals with the lowest serum titers showed some minor clinical symptoms (edema of eyelids and impairment of coordination). Recently, a non-toxic derivative of SLT-IIv was obtained by modification of the A part of the toxin by site-directed mutagenesis. This construct was used in preliminary immunization studies of 3- to 4-weeks-old pigs (Gordon et al., 1992). Two subcutaneous vaccinations with 50 μ g of this construct resulted in clinically normal pigs that had antitoxin antibodies in the serum. Successful protection studies were reported at the 12th International Pig Veterinary Society Congress (Whipp et al., 1992).

Active protection of pigs against edema disease aims at raising local antibodies in the intestine that have an anti-colonizing effect for the enterotoxemic pathogens, and/or that are capable of neutralizing SLT-IIv toxin before it enters the circulation. Eventually, circulating anti-toxin antibodies can neutralize SLT-IIv toxin in the bloodstream.

The incorporation of FedE and/or FedF into a vaccine composition as defined above, can lead to the production of antibodies capable of blocking the adhesion of F107 type fimbriated bacteria to the cell types to which they adhere *in vivo*, which could for instance also be found to include respiratory tract or urogenital tract infections.

According to another embodiment, the invention relates to the use of any of the polypeptides, fragments, or muteins, or antisense peptides thereof, as defined above for the preparation of a vaccine for preventing the occurrence of edema disease, postweaning diarrhea or any other disease state caused by the adhesion of F107 type fimbriated bacteria.

Additional examples of other possible infections which can be prevented by vaccination with the medicament according to this embodiment include urogenital tract infections as well as respiratory tract infections, caused by F107 type fimbriae carrying *E. coli* strains.

The present invention relates also to the use of the FedE and/or FedF polypeptides as defined above, for producing antibodies against a peptide of interest, comprising the steps of:

(a) insertion of a nucleic acid sequence, coding for the peptide of interest into the nucleic acid sequence encoding the *fedE* and/or *fedF* gene of a recombinant vector (more particularly pIH120) containing the *fedA*, *fedE* and/or *fedF* gene locus as defined above,

(b) transformation of the construct of (a) in suitable bacterial host cells,

(c) preparation of antibodies against the transformed strain,

(d) purification of antibodies specifically reacting with the antigen of choice according to methods known in the art.

The peptide of interest may be a peptide which is not immunogenic as such. By inserting its coding sequence into the above-mentioned "presentation vector", an antibody response may be obtained. Moreover, when administrated to live animals, the transformed host, expressing on its surface the modified F107 fimbriae which contain the peptide of interest, may induce a local immune response, leading to the production of antibodies useful for certain local therapeutic purposes.

In a first phase, the gene encoding the FedE/FedF adhesin can be adapted for incorporation of various epitopes by creating unique restriction sites using for instance PCR mutagenesis or the pMa/pMc system (Stanssens et al., 1989). Both techniques allow

the incorporation of epitopes in a directed fashion. Selection will then be based on intact fimbriation, combined with co-polymerization of the FedE/FedF adhesin carrying the suitable epitope. Undiminished adhesive property of the F107 fimbriae can be an additional advantage but is not essential for valorization of the obtained fusion adhesins. If adhesive power is lost, the simultaneous expression of adhesive fimbriae (not necessarily F107) together with the epitope-containing adhesin can be envisaged to obtain adhesive bacteria adhering to specific mucosal tissues. The tropism of these bacteria can be determined by the expressed fimbriae but not for the modified adhesin.

Incorporation of the adhesin in the F107 fimbriae (or any other fimbriae system) can be investigated by immunogold electron microscopy using specific antibodies directed against the foreign epitope and/or the FedE/FedF molecules.

Adhesion of fusion adhesins (incorporated in pili or not) can be investigated using isolated porcine intestinal villi, Caco2 cell lines and/or brush border membranes (BBM).

The obtained fusion vectors can be transformed in *E. coli* or *Salmonella* host strains and the local secretory IgA response, directed against the FedE/FedF and the foreign epitope, can be tested.

According to another embodiment, the present invention relates also to the use of the FedE and/or FedF polypeptides, muteins thereof, or fragments thereof, as defined above, for producing antibodies against a peptide of interest, comprising the steps of:

(a) chemically coupling the peptide of interest to *FedE* or *FedF*, or an *mutain* thereof, or a fragment thereof,

(b) raising antibodies against the polypeptide (or mutein, or fragment) - peptide complex.

The coupling procedure used in this embodiment may comprise any method known in the art.

According to this embodiment, FedE and/or FedF can be used as carrier molecules for immunization purposes.

The coupling can also take place on F107-fimbriated bacteria or bacteria carrying F107-related fimbriae containing the FedE and/or FedF polypeptides as defined above.

The invention also relates to a method for the *in vitro* detection of the capacity of a molecule to behave as a functional homologue or a ligand receptor with respect to

anyone of the polypeptides, or fragments thereof, or muteins thereof, as defined above, with said polypeptides, or fragments, or muteins, being liable to adhere to the cells to which they attach, assayed as defined above, comprising the steps of:

- contacting a molecule to be analyzed in the presence of a polypeptide, or fragment thereof, or mutein thereof, as defined above, with an antibody as defined above, and with said polypeptide, or fragment, or muteins, being preferably labeled, and with said antibody being able to bind specifically to the polypeptide, or fragment thereof, or mutein thereof;

- detecting the amount of labeled complex formed between the labeled polypeptide, or fragment thereof, or mutein thereof on the one hand, and the antibody on the other hand in the presence and in the absence of the molecule to be analyzed, with said molecule being liable to bind either on the same site (receptor) on the cell type to which the polypeptides, or fragments, or muteins attach *in vivo*, preferably the porcine small intestine wall, or on the specific site (epitope) FedE and/or FedF molecules on the F107 type fimbriated bacteria which mediates the binding between FedE and/or FedF and its receptor present on the tissue type on which these F107 type fimbriated bacteria attach *in vivo*;

- confirming the capacity of a molecule of which the addition to the components as described above results in a decrease of the amount of detectable labeled complex, as inhibiting the adhesion of F107 type fimbriated bacteria if the molecule inhibits the adhesion by at least 10%, and more preferably by at least 50%, as defined above.

The latter embodiment of the invention forms the basis for developing an *in vitro* drug screening system.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Schematic representation of plasmid pIH120 and derived constructs, and their phenotypic expression. (A) Relative positions of plasmids pIH2 and pIH120. The structural subunit gene *fedA* and the part of pIH120, represented in detail in B, is indicated. (B) Summary of the phenotypic expression of the constructs that were derived from plasmid pIH120. a. FedA shows on protein gel as two distinct bands.

Figure 2. Autoradiograph of the SDS-PAGE showing ^{35}S -cysteine labelled proteins after *in vitro* cell-free transcription-translation. Lane 1 represents pIH2; lane 2, pIH120; lane 3, pIH128; lane 4, pIH129; lane 5, pIH121; lane 6, pIH122; and lane 7, pIH126.

Figure 3A. Electron microscopy of negatively stained HB101 transformants containing plasmid pIH120. 1.5 cm represents 0.1 μm .

Figure 3B. Electron microscopy of negatively stained HB101 transformants containing plasmid pIH121. 1.5 cm represents 0.1 μm .

Figure 3C. Electron microscopy of negatively stained HB101 transformants containing plasmid pIH122. 1.5 cm represents 0.1 μm .

Figure 3D. Electron microscopy of negatively stained HB101 transformants containing plasmid pIH126. 1.5 cm represents 0.1 μm .

Figure 3E. Electron microscopy of negatively stained HB101 transformants containing plasmid pIH128. 1.5 cm represents 0.1 μm .

Figure 3F. Electron microscopy of negatively stained HB101 transformants containing plasmid pIH129. 1.5 cm represents 0.1 μm .

Figure 4. Western blot of bacterial extracts prepared from the transformants containing plasmid pIH120 and derived constructs. Proteins FedA and FedF are indicated. Lane 1 represents pUC18; lane 2, pIH120; lane 3, pIH121; lane 4, pIH122; lane 5, pIH123; lane 6, pIH126; lane 7 pIH128; lane 8, pIH129.

Figure 5A. Immune electron microscopy of transformants containing the native F107 genetic determinant pIH120. The F107 specific antiserum was absorbed to HB101(pIH126).

Figure 5B. Immune electron microscopy of transformants containing the F107-

gene cluster mutated in gene *fedF* pIH126. The F107-specific antiserum was absorbed to HB101(pIH126).

Figure 5C. Immune electron microscopy of transformants containing the cloning vector pUC18 (C). The F107-specific antiserum was absorbed to HB101(pIH126).

Figure 6. Nucleotide sequences of genes *fedE* and *fedF*. The amino acid sequences of the predicted proteins are shown below the nucleotide sequence. The amino acids constituting the putative signal sequences are indicated by negative numbers. The first amino acid sequence of the mature protein is numbered +1. This sequence was submitted to GenBank/EMBL and was assigned accession number Z26520.

Figure 7A. Nucleotide sequence comparison between F107 major subunit gene *fedA* and PCR fragments obtained with primer pair FedA 3 and FedA 4 on porcine *E. coli* strain 107/86 variants. The nucleotide sequence is given starting from the codon of the putative first amino acid of FedA (Imberechts et al., 1992b). The complete amino acid sequence of FedA is shown, together with the position of its amino acid residues. Only the nucleotides of the PCR fragments and the deduced amino acids that are different from *fedA* or FedA respectively, are indicated. The position of primer FedA 4 is indicated by underlining the corresponding nucleotides. The FedA nucleotide and amino acid sequence of respectively strains 107/86, S1274/88 and S221/90, S674/72 are indicated in SEQ ID NO 6 to 9.

Figure 7B. Nucleotide sequence comparison between F107 major subunit gene *fedA* and PCR fragments obtained with primer pair FedA 3 and FedA 4 on porcine *E. coli* strain 2134 variants. The nucleotide sequence is given starting from the codon of the putative first amino acid of FedA (Imberechts et al., 1992b). The complete amino acid sequence of FedA is shown, together with the position of its amino acid residues. Only the nucleotides of the PCR fragments and the deduced amino acids that are different from *fedA* or FedA respectively, are indicated. The position of primer FedA 4 is indicated by underlining the corresponding nucleotides. The FedA nucleotide and amino acid sequence of respectively strains 2134, 2171, 2173, 2203, 8199 and 8813 are indicated in SEQ ID NO 10-11, 12-13, 14-15, 16-17, 18-19 and 20-21.

EXAMPLES

EXAMPLE 1: ISOLATION AND CHARACTERIZATION OF THE COMPLETE SEQUENCE OF F107 FEDE AND FEDF GENES

1. Bacteria, media and constructions

E. coli strain HB101 was used to examine the phenotypic expression of the various recombinant constructs (Boyer et al., 1986). Transformants were grown for 20 h at 37 °C on Iso Sensitest Agar (Oxoid) supplemented with 100 mg/ml carbenicillin. Plasmids pIH2 and pIH20 were independent clones isolated from a genomic library of edema disease strain F107/86 of *E. coli* (serotype O139:K12(B):H1), coding for nonadhering and adhering F107 fimbriae, respectively (Bertschinger et al., 1990; Imberechts et al., 1992b). Both plasmids and the procedure of insertion inactivation of the cloned genes by a *Xba*I oligonucleotide (dCTAGTCTAGACTAG, Biolabs) were as described earlier (Imberechts et al., 1992b). The constructs used in this study and their phenotypes are presented in Table 1 and Fig. 1.

2. Phenotypic expression of fimbriae

The procedure of the electron microscopy and immune electron microscopy was described earlier (Imberechts et al., 1992b). For the preparation of specific FedF antiserum, the serum raised against isolated and purified F107 fimbriae was repeatedly absorbed at 4 °C with cultures of *E. coli* HB101(pIH126) until no anti-subunit activity could be demonstrated in Western blot. The preparation of Fab fragments of the F107-antiserum was done as described (Harlow & Lane, 1988). Fimbrial preparations of *E. coli* strains 107/86 and HB101(pIH120) were made by heating an overnight bacterial culture for 20 minutes at 65 °C. The supernatant of this heat extract was used in adhesion blocking tests. The preparation of bacterial extracts and Western blot, and the *in vitro* adhesion test were described earlier (Imberechts et al., 1992b).

Table 1.

Characteristics of plasmids pIH2, pIH120 and derived constructs.

Construct	Position of XbaI linker	Gene products encoded ^a	EMb	Adherence ^c
pIH2 ^d	/	p15,p16,p28,p75	+	-
pIH120	/	p15,p16,p28,p32,p75	+	+
pIH121	5.2 Kb	p15,p28,p32,p75	+	-
pIH122	5.2 Kb	p15,p28,p32,p75	+	-
pIH126	5.9 Kb	p15,p16,p28,p75	+	-
pIH128	6.3 Kb	p15,p16,p28,p75	+	-
pIH129	6.5 Kb	p15,p16,p28,p75	+	+

^a. As determined in a cell-free transcription-translation assay with ³⁵S-cysteine-labeled protein; p15 represents the fimbrial subunit FedA.

^b. Electron microscopy after negative staining

^c. Adherence *in vitro* to isolated porcine villi.

^d. Described earlier (Imberechts et al., 1992b).

3. In vitro transcription-translation assay

Large-scale preparations of plasmid DNA were made using the method of Qiagen (Qiagen Inc., Chatsworth, CA., US). To remove possible contaminating RNA, a second purification step by ultracentrifugation on CsCl was done (Maniatis et al., 1982). An *in vitro* transcription-translation system in the presence of ³⁵S-cysteine (*E. coli* S30 Coupled Transcription Translation System, Promega) was used. The assay was completely performed according to the instructions of the manufacturer. ³⁵S-labeled cysteine was purchased from Amersham. For electrophoresis, the Mini Protean II Dual Slab Cell system of Bio-Rad was used.

4. DNA sequencing and computer analysis

Subclones of plasmid pIH120 were made to determine the nucleotide sequence of both strands of the region about 500 nucleotides upstream of the restriction site for *NheI* of

pIH120, that is situated at position 5.2 Kb (Fig. 1). Sequencing was done using the dideoxy-termination reaction of Sanger with the universal and reverse sequencing primer for the pUC cloning vector (Sanger et al., 1977). Sequenase and Deaza T7 Sequencing Mixes (United States Biochemical Corporation) were used. The nucleotide sequence was analyzed by using the computer software The Gene Construction Kit (Textco, Inc.).

5. In vitro expression of the genes encoded by plasmid pIH120

Plasmids pIH2 and pIH120 code for non-adhering and adhering F107 fimbriae, respectively (Imberechts et al., 1992b). Since their sequences overlap for ca. 6 Kb (Imberechts et al., 1992b), and since plasmid pIH120 extended ca. 1.3 Kb more downstream from plasmid pIH2 (Fig. 1), it was concluded that plasmid pIH120 contained sequences which are essential for the receptor recognition of F107 fimbriae and which are not present in pIH2. Therefore, a nonsense linker was introduced in the unique restriction sites located in the distal half of construct pIH120. The resulting constructs are represented in Table 1 and Fig. 1. These mutants were examined for their expression of proteins and compared with plasmids pIH2 and pIH120.

In the cell-free *in vitro* transcription-translation test, construct pIH2 produced three major ³⁵S-cysteine labeled proteins, together with the β -lactamase protein encoded by the pUC18 cloning vector: p16, p28 and p75, with molecular mass of 16, 28 and 75 kilodaltons (kDa), respectively (Fig. 2). Two faint bands, one migrating somewhat slower and the other somewhat faster than p16 were also detected, and represent the structural protein FedA, respectively with and without signal sequence (Fig. 2, arrows). Plasmid pIH120 also coded for these proteins as well as for an additional band of 32 kDa, designated p32. Proteins p28, p32 and p75 appeared as double bands, suggesting that they were translated as precursor proteins with a signal sequence that was partially cleaved off, leading to the mature proteins.

Mutants pIH121 and pIH122 did not express protein p16 (termed FedE), indicating that gene p16 (fedE) is localized from 5.0 to 5.5 Kb. In mutants pIH126, pIH128 and pIH129 protein p32 (termed FedF) was not expressed, thus localizing the start codon of gene p32 (fedF) upstream of 5.9 Kb and extending to position 6.7 Kb (Fig. 1).

6. Expression of plasmids pIH120 and its derived constructs and analysis of their adhesive properties

The various constructs containing the *Xba*I linker insertions were transformed in *E. coli* HB101 cells to examine their phenotypic expression. Transformants carrying mutations in gene *fedE* or in gene *fedF* produced fimbriae as shown by electron microscopy (Fig. 3), indicating that neither FedE nor FedF is essential for the biosynthesis of F107 fimbriae. Both *fedE* mutants (HB101 with pIH121 or pIH122), and two out of three *fedF* mutants (HB101 with pIH126 or pIH128) produced fimbriae that were significantly longer than those of HB101 (pIH120) (Fig. 3 A to F). The fimbriae demonstrated on mutant HB101 (pIH129) resembled those of HB101(pIH120).

Bacterial extracts of transformants HB101(pIH120) and derived mutants were analyzed in Western blot using F107 antiserum (Fig. 4). A protein band of 15 kDa, representing the structural subunit FedA, was present in all samples, with the exception of HB101(pIH123). In addition, protein FedF was also detected by the antibodies in the extracts of the transformants harboring constructs pIH120, pIH121 and pIH122, but not in HB101(pIH126), HB101(pIH128) or HB101(pIH129). Since the F107 antiserum which was used in the Western blot was prepared against isolated and purified F107 fimbriae, it seems probable that FedF is a minor protein which is part of the fimbrial structure. To study this possibility more in detail, the F107 antiserum was adsorbed with *E. coli* strain HB101(pIH126), which produced F107 fimbriae without FedF. The resulting serum was largely purged from antisera directed against Fed A and now contained mainly antibodies directed against protein FedF as was indicated by Western blot (data not shown), and by immune electron microscopy. As shown in Fig. 5A to 5C, the gold grains were attached to the fimbriae formed by *E. coli* strain HB101(pIH120). The antiserum did not bind to the fimbriae produced by strain HB101(pUC18) nor by HB101(pIH126).

The adhesive capacities of the various transformants were demonstrated by the *in vitro* attachment to isolated porcine villi. In contrast to strain HB101(pIH120), strain HB101(pIH2) did not adhere to the isolated villi, as was shown earlier (Imberechts et al., 1992b). Neither HB101(pIH121) and HB101(pIH122), nor HB101(pIH126) and HB101(pIH128) bound to the villi. Only strain HB101(pIH129) attached *in vitro* (Fig. 1). In conformity with the adhesive capacity of *E. coli* strain 107/86, the attachment of the various transformants to the isolated villi was not sensitive to 100 mM mannose.

7. The nucleotide sequences of genes *fedE* and *fedF*, and the primary amino acid sequences of proteins FedE and FedF

The nucleotide sequence of plasmid pIH120 (Fig. 1) was determined between approximately 500 nucleotides upstream of restriction site *NheI* (position 5.2 Kb) and *EcoRI* (7.3 Kb). Two large open reading frames were found (Fig. 6). One open reading frame of 900 nucleotides contained the sites *BclI*, *SnaBI* and *BamHI*, which were used to insert the oligolinker *XbaI*, resulting in constructs pIH126, pIH128, and pIH129, respectively. Therefore, it was acknowledged that this open reading frame contained gene *fedF*. The gene product FedF had a calculated molecular mass of 32.637 Da, and contained a hydrophobic N-terminal end of 20 amino acids which has characteristics of a signal peptide (Oliver, 1985). Consequently, the mature form of FedF is 30.341 Da, which is in good agreement with the size of the protein on SDS-PAGE.

Table 2.

Amino acid sequence homology in % between FedE and FedF and various minor fimbrial subunits

	FimF	FimG	FimH
FedE	32.2	29.3	29.2
FedF	33.0	32.3	28.0
	PapE	PapF	PapG
FedE	29.5	33.1	34.5
FedF	35.8	31.3	27.3
	SfaG	SfaS	SfaH
FedE	24.0	30.7	29.8
FedF	38.5	40.5	28.9
	F17-G		
FedE	35.7		
FedF	32.0		

Another open reading frame was localized immediately upstream of *fedF*, and coded for a protein of 18.402 Da. In this open reading frame, the restriction sites *NheI* and *StuI* were localized. Since the insertion of the nonsense linker in these unique sites lead to constructs pIH122 and pIH121, respectively, which did not code for FedE, it can be concluded that this open reading frame contains gene *fedE*. A signal sequence of 22 amino acids was identified, and the processed protein has a calculated mass of 15.949 Da, in agreement with the protein gel analysis of the cell free transcription-translation test. The primary structure of FedE shows some homology to the minor fimbrial subunits of Pap, type 1 and S fimbriae, and especially to the adhesins PapG (34.5%) and F17-G (35.7%). The deduced amino acid sequences of FedE and FedF were aligned with minor subunits of Pap, type 1, S and F17 fimbriae (Table 2). The calculated homologies (similarities) of the proteins with these fimbrial systems of different origins (strains associated with urinary tract infections in humans, pathogenic and apathogenic *E. coli*, meningitis causing bacteria, and bovine ETEC strains, respectively) were between 27 and 40%. The highest general similarity (40%) was found to be between FedF and adhesin SfaS, although alignment of these proteins did not reveal clusters of higher local similarity (homology).

Based on the calculated homology (40.5%), it seems attractive to conclude that protein FedF is related to adhesin SfaS. However, the receptor binding site of protein SfaS was identified and was demonstrated to bear homology to that of the sialic acid binding proteins K99 adhesin, CFAI adhesin, cholera B subunit and *E. coli* LTI B subunit (Finkelstein et al., 1987; Karjahlainen et al., 1989; Klemm, 1982; Leong et al., 1985; Morschhäuser et al., 1990; Roosendaal et al., 1984). No sequence was identified in FedE or in FedF which could be aligned with this conserved sialic acid binding sequence. In the same way, although the comparisons of FedE with PapG and of FedE with F17-G suggested certain homologies (34.5% and 35.7%, respectively), this could not be confirmed by alignment of the amino acid sequences. Therefore, this kind of calculations seem to be of only a limited value in determining the function of FedE and FedF.

8. FedF specific monovalent antiserum

A polyclonal F107-antiserum (Imbrechts et al., 1992b) was raised in rabbits by using purified F107 fimbriae as an antigen. To obtain FedF specific antiserum, the polyclonal F107 serum was absorbed with *E. coli* strain HB101 (pIH126), producing F107-fimbriae which lack protein FedF. The technique of serum absorption consisted of the

incubation of the polyclonal serum with the HB101(pIH126) bacterial suspension for 4 hours at 4°C. This absorption was repeated until this absorbed serum detected only protein FedF in Western blot.

EXAMPLE 2: CHARACTERIZATION OF FEDA GENES OF F107 TYPE FIMBRIATED STRAINS

1. Bacterial strains.

The *Escherichia coli* strains used in this study were isolated from pigs with edema disease or postweaning diarrhea and belong to various serotypes (Table 3). All strains are known to express fimbriae that are different from F4, F5, F41 and 987P after growth in vitro, and were kindly provided by the various authors (Casey et al., 1992, Kennan and Monckton, 1990; Nagy et al., 1992; Rippinger, 1993; Salajka et al., 1992).

The *E. coli* strains that were used as controls for the amplification of genes coding for the various virulence factors were the following: 107/86 (FedA and SLT-IIv[B]) (Bertschinger et al., 1990), "Abbottstown" (LTI) (Sojka, 1971), B41 (STIa), G7 (LTI and STII) (Sojka, 1971), and HB101 (negative control) (Boyer and Roulland-Dussoix, 1969). For PCR analysis, the bacteria were grown in LB medium (Miller, 1972) for 20 h at 37°C.

2. PCR and cloning of *fedA*-like genes.

The isolates were screened for the presence of *fedA* sequences by means of PCR with primers FedA 1 and FedA 2 as described earlier (Imberechts et al., 1992b). For PCR cloning, new oligonucleotide primers FedA 3 and FedA 4 were synthesised on a PCR Mate 391 DNA synthesizer (Applied Biosystems). The sequences of primers FedA 3 and FedA 4 were deduced from FedA 1 and FedA 2, and contained restriction sites. The sequences of FedA 3 and FedA 4 (5' to 3') were GC TCT AGA GCA AGC TTT GTG AAA AGA CTA GTG TTT ATT TC (SEQ ID NO 22) and GC TCT AGA GCG AAT TCT CTT GTA AGT AAC CGC GTA AGC (SEQ ID NO 23) respectively, and were used in a final concentration of 1 mM each. *Pfu* DNA polymerase and buffer #2 (Stratagene) were used according to the supplier's information. The dNTP were purchased from USB (United States Biochemicals) and used in a final concentration of 200 mM each. Boiled

bacteria were used as DNA template. To this end, approximately 10^9 bacteria that were grown in LB were harvested by centrifugation. The bacterial pellet was resuspended in 1 ml of sterile water and subsequently boiled for 10 minutes. After centrifugation, 5 ml of the supernatant was used in the PCR in a final reaction volume of 50 μ l. The PCR set-up was as follows: 4 min at 94°C, 25 cycles amplification (1 min 94°C, 1 min 50°C, and 2 min 75°C), and 10 min at 75°C for final polymerisation. The amplified products, which had a calculated length of 544 bp, were put on 1.5% TAE agarose gel (Maniatis et al., 1982), the bands were cut from the gel, isolated with Geneclean II kit (Bio 101), and cloned with the use of restriction sites *EcoRI* and *HindIII* in cloning vector pUC19 (Yanisch-Perron et al., 1985).

3. DNA Sequence analysis.

The cloned PCR products were sequenced with Sequenase® T7 DNA Polymerase of USB with the use of pUC sequencing primers. The resulting sequences were deposited in the Genbank collection. GenBank accession numbers are: L26104 (strain 2203), L26105 (strain 8813), L26106 (strain 8199), L26107 (strain 2134), and L16108 (strain S22/90).

4. Screening of *E. coli* isolates for *fedA*.

As a first screening, the porcine postweaning and edema disease strains presented in Table 3 were examined by PCR with primers FedA 1 and FedA 2, specific for gene *fedA* of enterotoxemic strain 107/86 (Imberechts et al., 1992b). A band of 510 base pairs, corresponding to the *fedA* sequence, was demonstrated in all these isolates (data not shown). Digestion of the amplification products with restriction enzymes *HpaI*, *NsiI* and *SmaI*, for which sites exist in gene *fedA* of strain 107/86 (Imberechts et al., 1992b), demonstrated that these sites were also present in the amplified sequences. Therefore, it seemed that gene *fedA* of edema disease strain 107/86 or *fedA* related genes were detected in the pathogenic isolates.

5. Sequencing of PCR products.

To further characterise the *fedA* sequences in the various *E. coli* strains, PCR was done on the same isolates but with primers FedA 3 and FedA 4. *HindIII*, *EcoRI*-digested PCR products were ligated into vector pUC19 digested with the same enzymes. From each of the cloning experiments one clone was selected for sequencing. The

nucleotide sequences of these inserts are presented in SEQ ID NO 6, 8, 10, 12, 14, 16, 18 and 20. The results are presented in Figure 7 and Table 3, and show that six different sequences were found, including *fedA*, the major fimbrial subunit gene of *E. coli* strain 107/86. The strain in which gene *fedA* was identified originated from a case of edema disease. Almost always the nucleotide differences result in amino acid differences (as presented in SEQ ID NO 7, 9, 11, 13, 15, 17, 19 and 21). In two exceptions the change of the third nucleotide of the codon triplet (positions 83 and 90) does not influence the amino acid residue translation. Alignment of the six nucleotide and amino acid sequences showed that they are identical for at least 95% and 91% respectively. Therefore, the newly found sequences and the *fedA* gene are very closely related.

Four sequences contain three additional nucleotides as compared to the *fedA* gene, and therefore two groups of *fedA*-related sequences can be distinguished. One group (SEQ ID NO 6 and 8) consists of sequences that are clearly identical to gene *fedA* since only two nucleotide mismatches were found. The sequences of the other group (SEQ ID NO 10, 12, 14, 16, 18 and 20) differ somewhat more from *fedA*, especially by the CCG triplet inserted between the amino acid 101 and 102 codons of protein FedA. The variation between the four sequences of the second group is limited: only at five places nucleotide differences were identified. The nucleotide sequences show that both groups can be differentiated by the digestion of the PCR products with restriction enzyme *Ngo*MI. The proline-encoding CCG triplet is part of the restriction site recognised by this enzyme (GCCGGC) (SEQ ID NO 24) (data not shown). Six additional *E. coli* strains 8804, 8810, 8872, 8877, 8923 and 8984, which were described to express the same type of colonisation factor as strain 8813 (Salajka et al., 1992), and two fimbriae-bearing Australian postweaning strains (Kennan and Monckton, 1990) were examined by PCR with primers FedA 3 and FedA 4. Subsequent *Ngo*MI digestion of the amplified product demonstrated that the *fedA*-related sequences of these strains contain the additional CCG triplet.

It seems that a family of *fedA*-related genes can be found, similar to the variation which is known in the K88 fimbrial subunit gene (Dykes et al., 1985). Consequently, the *fedA*-related sequences that we present here are the nucleotide sequences of the major subunit genes of the recently described adhesive fimbriae 2134P, "8813" and of those detected on German postweaning diarrhea strains. We conclude that the colonisation factors identified on Australian, Czech, German, Hungarian and Swiss isolates are related to one another, and that they belong to the F107 family of fimbriae.

The proteins encoded by the *fedA*-related sequences that contain the additional CCG codon (SEQ ID NO 11, 13, 15, 17, 19 and 21) differ from subunit FedA (SEQ ID NO 7 and 9) in 9 to 12 amino acids. This different amino acid composition, including the additional proline residue, may account for distinct serological epitopes of the proteins, both linear and conformational. In this way, earlier described results can be explained that were obtained with immunological techniques. Nagy et al. (1992) demonstrated that serological methods could distinguish between 2134P pili and F107 fimbriae. Polyclonal anti-F107 serum reacted with both fimbriae, whereas anti-2134P monoclonal antibodies only recognised 2134P (Nagy et al., 1992). In another study, polyclonal, cross-absorbed antisera raised against fimbriae expressed by *E. coli* strains 107/86, S674/72, 2134, 2203, and 8199, were used in immunological experiments (Rippinger, 1993). The study showed that these fimbrial antigens could be classified in "ab" (strains 107/86 and S674/72) and "ac" variants (strains 2134, 2203 and 8199). This classification refers to that of K88 antigens (Guinee and Jansen, 1979) where "a" schematically represents an epitope common to both variants, and "b" and "c" refer to epitopes specific for either one of them. *E. coli* strains 107/86 and 2134 belong to variants "ab" and "ac" respectively. It is clear that serological techniques and nucleotide sequence analysis have demonstrated in parallel the existence of a F107 family of fimbrial antigens that are associated with edema disease and postweaning diarrhea.

Table 3. Porcine *E. coli* strains examined in this study

Strain name	Serogroup	Pathology ^a	Fimbrial type ^b	Reference
2134	O157:H19	PWD	2134P	Casey et al., 1992, Nagy et al., 1992
2171	O141:H4	PWD	2134P	Casey et al., 1992
2173	O147	PWD	2134P	Nagy et al., pers. comm.
2203	O138	PWD	2134P	Nagy et al., pers. comm.
8199	O141:K85ab:H4	PWD	F107"ac"	Rippinger, 1993, Wittig et al., pers. comm.
S221/90	O138:K81	PWD	F107"ab"	Wittig et al., pers. comm.
S1247/88	O139:K82	ED	F107"ab"	Wittig et al., pers. comm.
S674/72	O157:K-	ED	F107"ab"	Wittig et al., pers. comm.
8813	O147:F18	PWD	"8813"	Salajka et al., 1992
Av24	O141:K85ab	PWD	-	Kennan & Monkton, 1990
2253	O141:K85ab	PWD	-	Kennan & Monkton, 1990

^a PWD: postweaning diarrhea, ED: edema disease^b As defined by the author by means of serological techniques

EXAMPLE 3 : CHARACTERIZATION OF FEDE AND FEDF GENES OF F107 TYPE FIMBRIATED STRAINS

1. Bacterial strains

The *Escherichia coli* strain 2134 used in this study is described in example2 and table 3. Control strains were also as described in example2.

2. PCR and cloning of *fedE* and *fedF* like genes.

For the amplification of the *fedE* and the *fedF* gene of *E. coli* strain 2134, two primer pairs were designed and custom synthesized (Pharmacia, Brussels). The forward primer for the *fedE* gene is biotinylated at the 5'-end. The forward primer for the amplification of *fedE* gene is designated FedE 1 and contains an *EcoR*I restriction site at its 5'-end. The backward primer is FedE 2. The sequence of the FedE 1 primer (5' to 3') and starting with biotine (indicated as X) is as follows: X GCG GAA TTC CCT AAA ATT GCA TTG ATT GCG (SEQ ID NO 24). The FedE 2 primer is (from 5' to 3'): TAA GCA CCA GAC AGA TTA AAA TCC GC (SEQ ID NO 25).

The primers for the amplification of the *fedF* gene are designated FedF 14 and FedF 15 the sequences of which are (from 5' to 3') TGG AAA CTA CTG GGA TAA (SEQ ID NO 26) and CAA AAT ACC TCG CAC TCA T respectively (SEQ ID NO 27).

The preparation of the template DNA was as described in example 2 and the PCR reaction conditions were also as described in example2 except that for the *fedE* and the *fedF* gene the elongation temperature was set at 55°C.

The PCR amplified fragments were gel purified as described in example2.

Sequence analysis of the *fedE* fragment was performed on the PCR material without molecular cloning using the FedE primers described above to prime the sequencing reactions.

The *fedF* PCR fragment was treated with restriction endonucleases *Bcl*I and *Sac*I to allow cloning into the plasmid pUC19, opened with *Bam*H1 and *Sac*I. The recombinant plasmid was then used for sequencing, the reactions of which were primed with the universal and reverse primers.

3. Sequence analysis.

Sequence analysis was performed on a model A373 Applied Biosystems sequencer using the dye-labeled termination technology.

For the *fedE* gene the primers used for PCR amplification were used to prime the sequencing reactions directly on the purified PCR fragment.

For the *fedF* gene, the PCR fragment was cloned in the pUC19 vector and sequences were determined using the pUC sequencing primers. To obtain the complete sequence of the fragment, subclones were made from the original plasmid. Therefore, the plasmid was cut with *Hind3* and *Xho1*, the sites were filled with klenow polymerase and the plasmid was closed. Alternatively, the plasmid was opened with *BamH1* and *Sac1*, ends were blunted and the plasmid was closed with ligase. Both deletion plasmids were used to complete the sequence of the *fedF* gene, using again the pUC sequencing primers.

4. Sequence comparison.

The sequence obtained from the PCR fragment encoding *fedE* of strain 2134 was completely identical with the sequence of the *fedE* gene of the *E. coli* strain F107/86 as described in SEQ ID NO 4.

The sequence of the *fedF* gene of *E. coli* strain 2134 differs from the *fedF* gene of strain F107/86 as described in SEQ ID NO 5. The nucleotide changes also result in changes in the encoded polypeptide. Alignment of the *fedF* genes shows similarity of more than 98% on the nucleotide level as well as on the amino acid level.

These results indicate that differences in the minor subunits of the pili of strains associated with pig edema disease (F107/86) or with postweaning diarrhea can occur although to a limited extent.

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CLAIMS

1. Composition comprising at least one of the polypeptides, termed respectively FedE and FedF, in a substantially pure form, with said FedE and FedF having a respective molecular mass of approximately 16 and 32-kDa as determined on Western blot, and with said FedE and FedF:

- * each being present in F107 type fimbriae in a minor amount which constitutes approximately 1 % of the total protein content of F107 type fimbriae; and,

- * each being nonessential to the normal assembly of F107 type fimbriae; and,

- * both being essential for the adhesion of F107 type fimbriae to porcine small intestine;

or a composition comprising fragments of FedE and/or FedF, or muteins thereof, insofar that said fragments and said muteins have retained the properties of the polypeptides as described above; and with said muteins consisting of substitutions and/or deletions and/or additions of one or several amino acids.

2. Composition comprising at least one of the polypeptides FedE or FedF according to claim 1, with said FedE and/or FedF being characterized by their respective amino acid sequences as shown in SEQ ID NO 1 and 2.

3. Polypeptide FedE characterized by its amino acid sequence as shown in SEQ ID NO 1.

4. Polypeptide FedF characterized by its amino acid sequence as shown in SEQ ID NO 2.

5. Polynucleic acid in substantially isolated form constituted by, or comprising a contiguous sequence of at least 10 nucleotides selected from:

- (a) the polynucleic acids which code for any of the FedE or FedF polypeptides according to any of claims 1 to 4,

- (b) the polynucleic acid sequences which hybridize to any of the polynucleic acids as defined in (a), or,

(c) the polynucleic acid sequences which are degenerate as a result of the genetic code to the polynucleic acid sequences as defined in (a) or (b) and which either code for a polypeptide according to any of claims 1 to 4, or hybridize to a polynucleic acid which codes for a polypeptide according to any of claims 1 to 4, more particularly the polynucleic acid shown in SEQ ID NO 3, and more preferably those containing the FedE and/or FedF coding regions as shown in SEQ ID NO 4 and 5.

6. Probe containing at least 10 nucleotides, preferably containing from about 15 to about 50 nucleotides, with said probe being able to detect the presence of a polynucleic acid according to claim 5, or the complementary sequence thereof, in a biological sample, by hybridizing to a target region present in a polynucleic acid sequence according to claim 5, more particularly to a polynucleic acid sequence contained in any of SEQ ID NO 3, or SEQ ID NO 4, or SEQ ID NO 5; and with said probe being possibly chemically modified.

7. Recombinant vector, particularly for cloning and/or expression, with said recombinant vector comprising a vector sequence, and at least a part of any of the polynucleic acid sequences as defined in claim 5, more particularly SEQ ID NO 3, SEQ ID NO 4 or SEQ ID NO 5, and further characterized in that the coding sequence is operably linked to control sequences capable of providing for the expression of the coding sequence by the specific host.

8. Bacterial host with a recombinant vector according to claim 7, particularly a plasmid, or a phage, in which a polynucleic acid according to claim 5 coding for one of the polypeptides according to anyone of claims 1 to 4.

9. Recombinant polypeptide expressed by culturing a suitable bacterial host as defined in claim 8 which is transformed with a recombinant vector as defined in claim 7, with said recombinant polypeptide being possibly part of a fusion protein, consisting of any nonhomologous protein, chosen to optimize the expression of the polypeptide.

10. Method for producing a recombinant polypeptide according to claim 9, comprising incubating a host cell as defined in claim 8 under conditions that provide for

the expression of the coding sequences.

11. Antibody characterized by being specifically directed against any of the polypeptides, muteins thereof, or fragments thereof, as defined in any of claims 1 to 4, with said antibodies being preferably monoclonal antibodies; or antigen-binding fragment thereof of the F(ab')₂, F(ab) or single chain Fv type or any type of recombinant antibody derived thereof.

12. Antibody, or antigen-binding fragment of the F(ab')₂, F(ab), single chain Fv or all other types of recombinant antibodies derived therefrom, as defined in claim 11, further characterized in that it can inhibit the adhesion of F107 type fimbriated bacteria to the specific cell type to which they attach *in vivo*.

13. Method for detecting the presence of F107 type fimbriated bacteria in a sample liable to contain it, comprising the steps of:

- contacting the sample to be analyzed with antibodies as defined according to claim 11 or 12, appropriately labeled,
- detecting the presence of the labeled antigen-antibody complex formed.

14. Method for detecting the presence of bacteria containing F107 type fimbrial *fedE/fedF* gene clusters, in a sample liable to contain these bacteria, comprising the steps of:

- possibly performing a PCR amplification step with suitable primers,
- contacting either the polynucleic acids of the sample, appropriately released, or the amplified PCR product with a probe, as defined in claim 6, liable to target either the polynucleic acid sequences according to claim 5, and particularly the polynucleic acids as shown in SEQ ID NO 3 to 5, or a nucleic acid sequence showing at least 50 to 60% homology, preferably more than 70 to 80%, more preferably more than 90 to 95%, and most preferably more than 98 to 99% homology to the sequences shown in SEQ ID NO 3 to 5.

15. Antisense peptides prepared against any of the polypeptides, or fragments thereof, or muteins thereof, as defined in any one of claims 1 to 4.

16. Pharmaceutical composition containing or consisting of at least one of the polypeptides, or fragments thereof, or muteins thereof, according to any one of claims 1 to 4 or any of the antibodies as defined in claim 11 or 12 or any of the antisense peptides as defined in claim 15.

17. Use of any of the polypeptides, fragments thereof, or muteins thereof, or antibodies, or antisense peptides, as defined in claims 1 to 4, 11, 12 and 15, for the preparation of a medicament for treating edema disease, postweaning diarrhea, or any other disease states caused by the adhesion of F107 type fimbriated bacteria.

18. Vaccine composition containing or consisting of at least one of the polypeptides, or fragments thereof, or muteins thereof as defined according to any one of claims 1 to 4, and possibly also polypeptide B of the toxin of SLT-IIv, or the toxoid, with said polypeptides or fragments thereof, or muteins thereof, being able to raise antibodies capable of blocking the adhesion of F107 type fimbriated bacteria to the cells to which they attach, or of raising toxin-inactivating (or neutralizing) antibodies.

19. Use of any of the polypeptides, fragments, or muteins, or antisense peptides thereof, as defined according to any one of claims 1 to 4, for the preparation of a vaccine for preventing the occurrence of edema disease, postweaning diarrhea or any other disease state caused by the adhesion of F107-fimbriated bacteria.

20. Use of a polypeptide according to any of claims 1 to 4, for producing antibodies against a peptide of interest, comprising the steps of:

(a) insertion of a nucleic acid sequence, coding for the peptide of interest into the *fedE*-and/or *fedF* polynucleic acid according to claim 5, possibly contained in a recombinant vector as defined in claim 7,

(b) transformation of the construct of (a) in suitable bacterial host cells,

(c) preparation of antibodies against the transformed strain,

(d) purification of antibodies specifically reacting with the antigen of choice.

21. Use of a polypeptide according to any one of claims 1 to 4, for

producing antibodies against a peptide of interest, comprising the steps of:

- (a) chemically coupling the peptide of interest to any of the polypeptides according to claims 1 to 4,
- (b) raising antibodies against the resulting polypeptide-peptide complex.

22. Method for the *in vitro* detection of the capacity of a molecule to behave as a functional homologue, or a ligand receptor with respect to anyone of the polypeptides, or fragments thereof, or muteins thereof according to anyone of claims 1 to 4, with said polypeptides, or fragments, or muteins, being liable to adhere to the cells to which they attach, comprising the steps of:

- contacting a molecule to be analyzed in the presence of a polypeptide, or fragment thereof, or mutein thereof, as defined according to anyone of claims 1 to 4, with an antibody according to claim 11 or 12, and with said polypeptide, or fragment, or muteins, being preferably labeled, and with said antibody being able to bind specifically to the polypeptide, or fragment thereof, or mutein thereof;
- detecting the amount of labeled complex formed between the labeled polypeptide, or fragment thereof, or mutein thereof on the one hand, and the antibody on the other hand in the presence and in the absence of the molecule to be analyzed, with said molecule being liable to bind either on the same site (receptor) on the cell type to which the polypeptides, or fragments, or muteins attach *in vivo*, preferably the small intestine wall, or on the specific site (epitope) of the FedE and/or FedF molecules on the F107 type fimbriated bacteria which mediates the binding between FedE and/or FedF and its receptor present on the tissue type on which these F107 type fimbriated bacteria attach *in vivo*;
- confirming the capacity of a molecule of which the addition to the components as described above results in a decrease of the amount of the detectable labeled complex, as inhibiting the adhesion of F107 type fimbriated bacteria provided the molecule inhibits the adhesion by at least 10%, and more preferably by at least 50%.

1/18

A

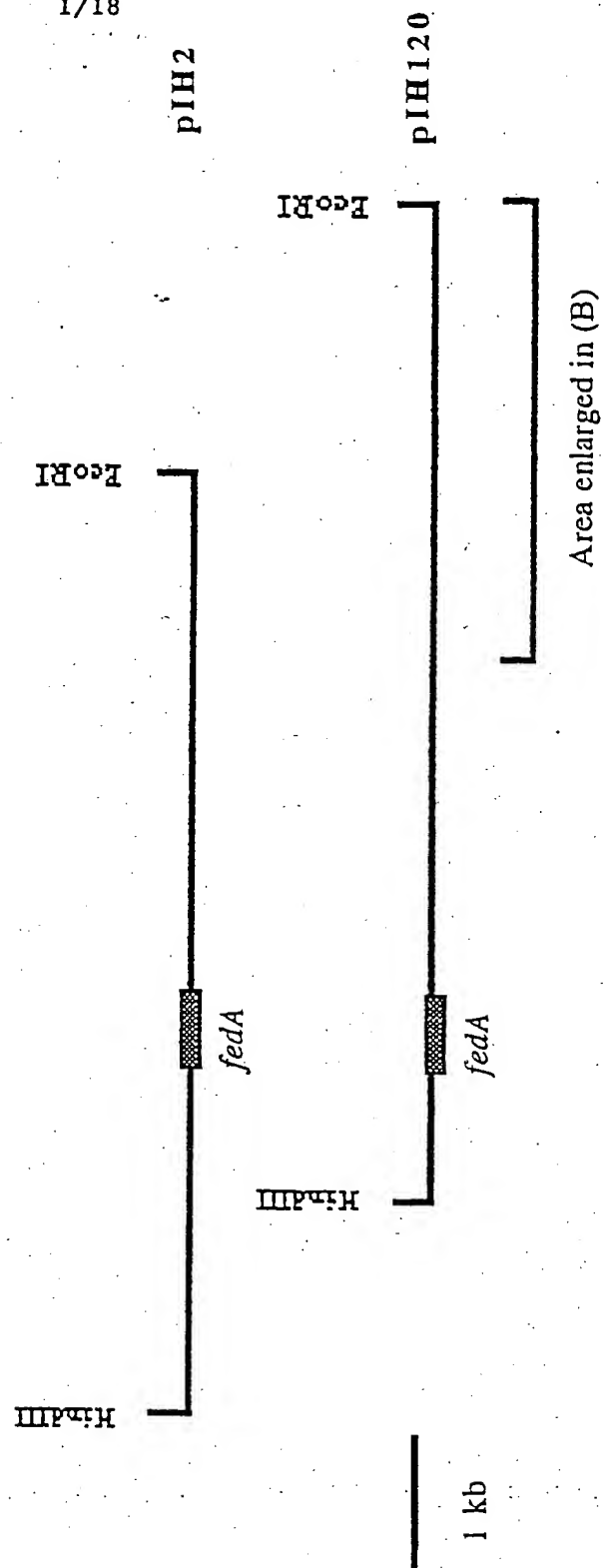
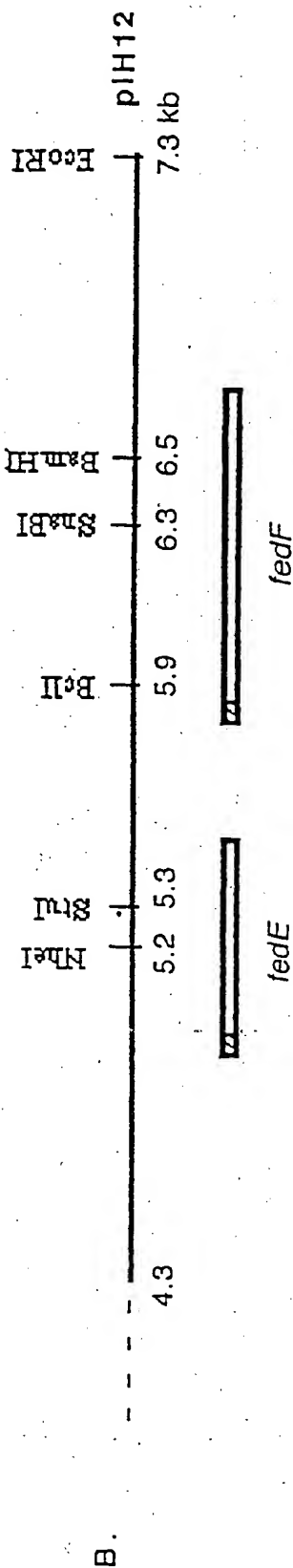


Figure 1

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2/18



pIH mutant:

Electron

microscopy Fimbriae:

Adhesion test *in vitro* :*In vitro* translation with ³⁵S-cysteine:

75 kDa

32 kDa

28 kDa

16 kDa

FedA*

Bacterial extract:

32 kDa

15 kDa

122 121

126

128 129

+

+

+

+

-

-

-

-

+

+

+

+

+

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3/18

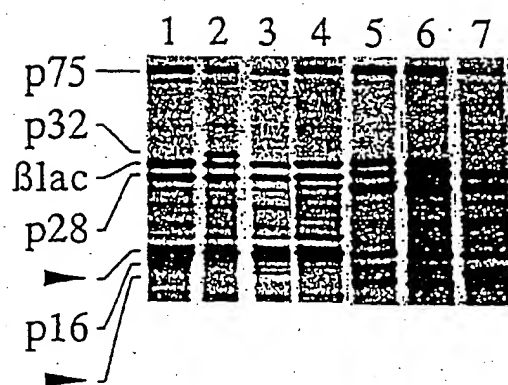


Figure 2

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4/18

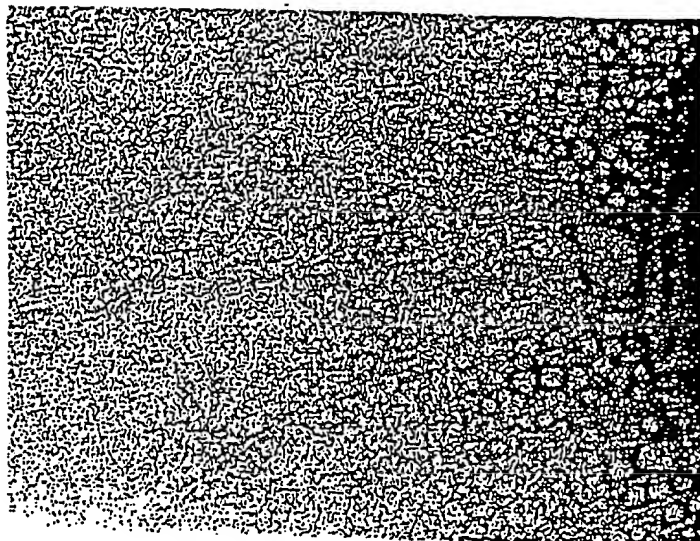


Figure 3A

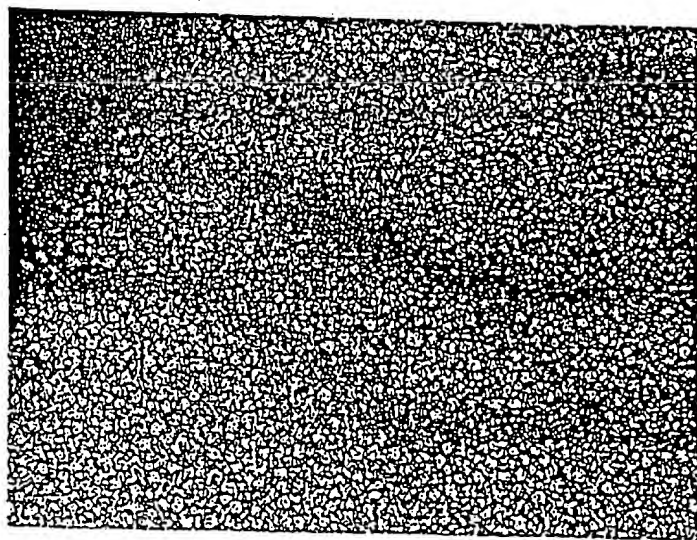


Figure 3B

5/18

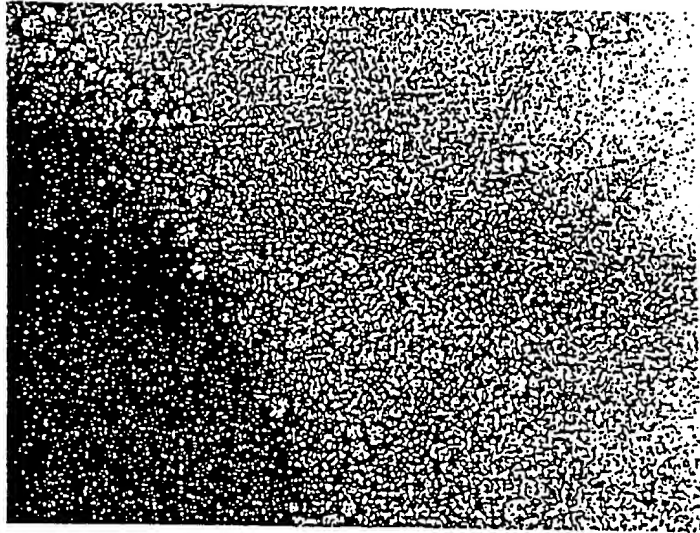


Figure 3 c

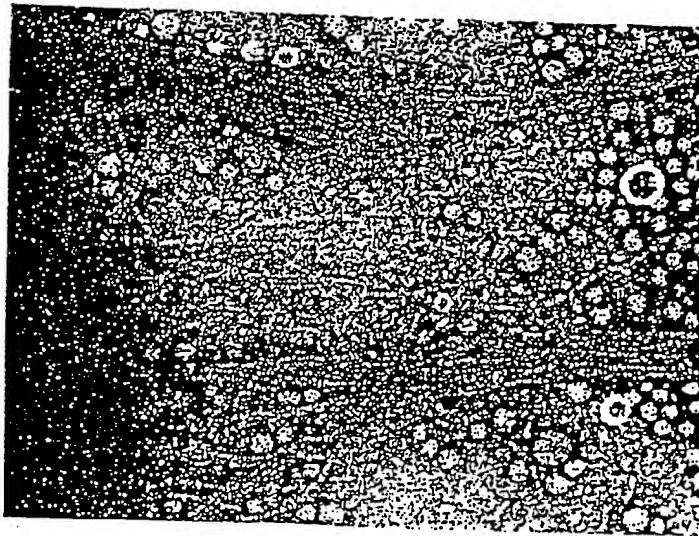


Figure 3 D

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6/18

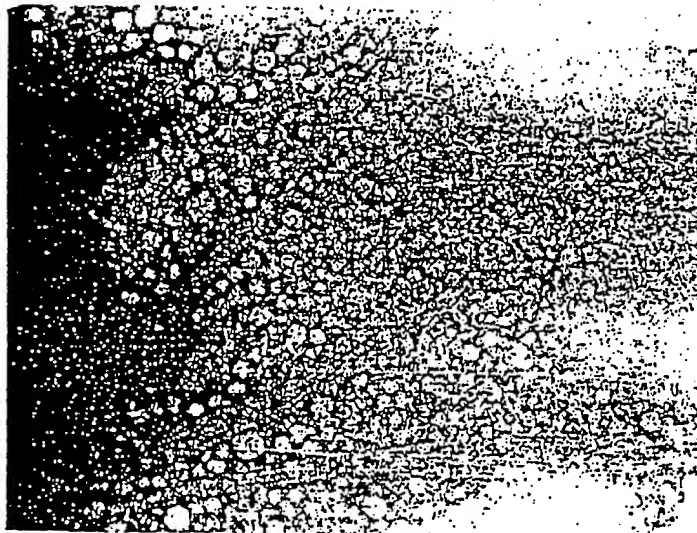


Figure 3 E

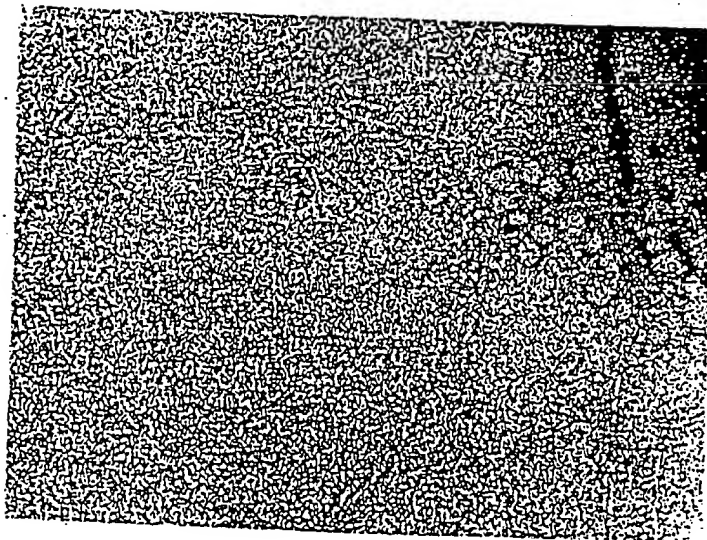


Figure 3 F

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7/18

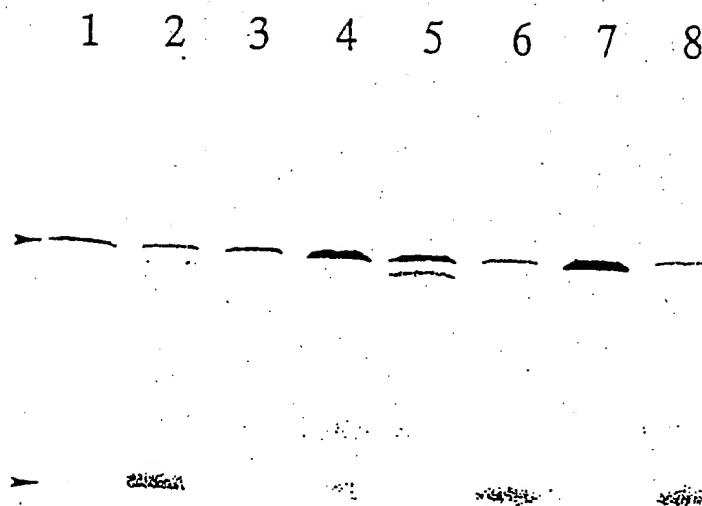


Figure 4

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8/18

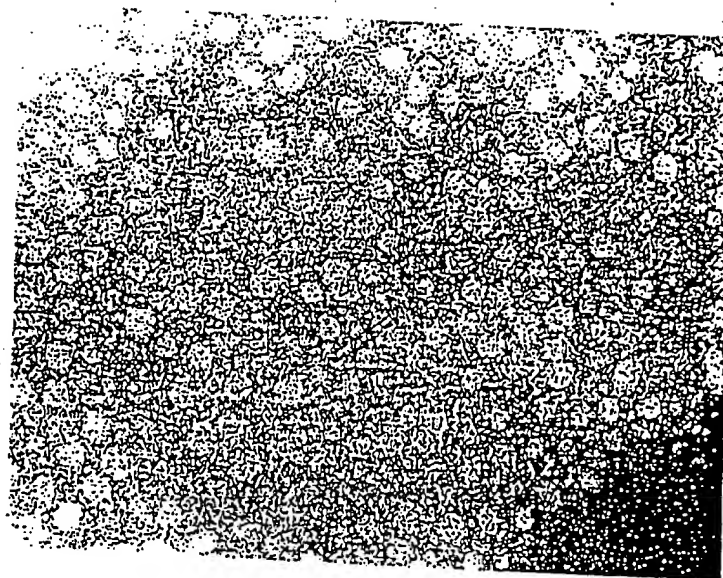


Figure 5A

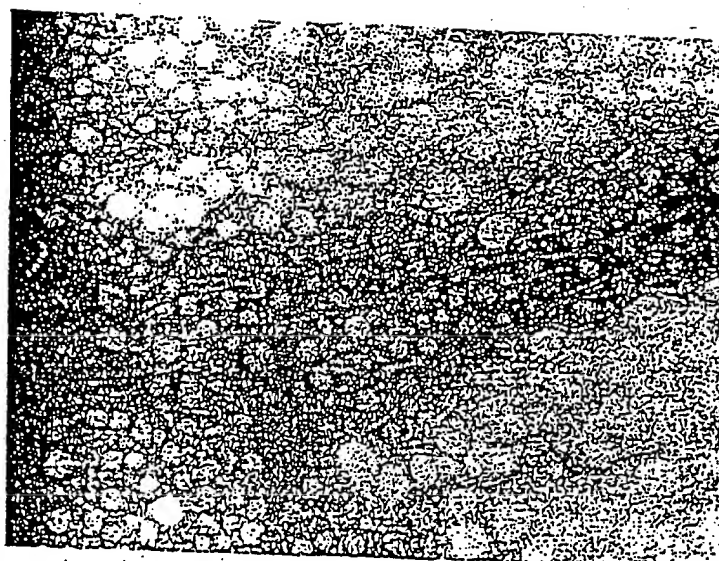


Figure 5B

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9/18

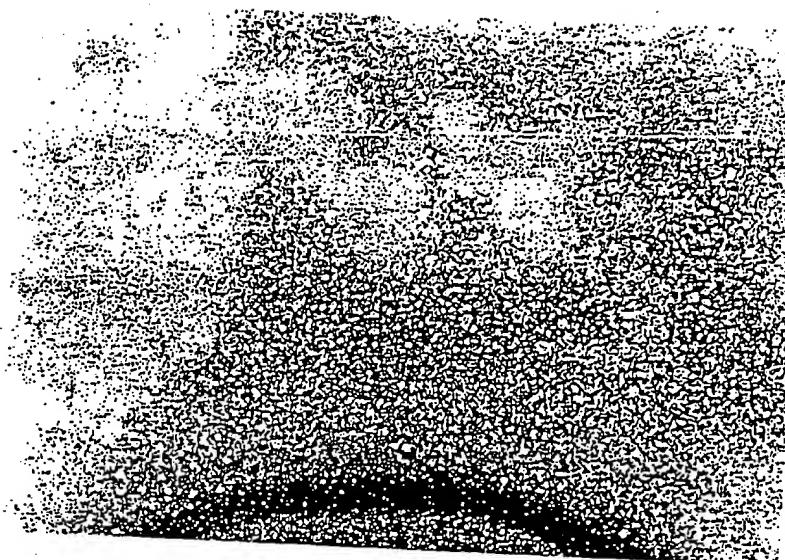


Figure 5 c

Fig. 6

10/18

330 340 350 360 370 380
CCGCCCTGCCTCCATTAAGAATGGcGTCAAGATGCAGAAAAACAGCTAAAGATGGAACTA

390 400 410 420 430 440
CGTGGAGACACAACTTGGTTGAAGAATCCAACACCGTACTACATGGCTATTATTAGTGTGA

450 460 470 480 490 500
AAACATGATGGTAAAGATATTTCCCTCAGTGATAATGTGATGAAAGAAATCGCACAACTT

510 520 530 540 550 560
AGGCCATTTAGTGATGTGAATTTAGGTAGAGTGGTACGTGATAAAATTAGTGTAGATGCC

570 580 590 600 610 620
GTAAATGACTGGGGAGGGTTCAGAGTTATGAAATTCCTAAAATTGCATTGATTGCGTT
METLysPheProLysIleAlaLeuIleAlaLeu

630 640 650 660 670 680
GATAATGGTGATGATGATTAGTGTGATGTGGAGGCAACAACTCTACTACTGCAGTGCTGAC
IleMETValMETMETIleSerAspValGluAlaThrAsnSerThrThrAlaValLeuThr

690 700 710 720 730 740
GATTAACGTACCTTCACCCCGCCGAGTTGCGATATTAAGATGCCTTCCTCATATGATTT
IleAsnValThrPheThrProProSerCysAspIleLysMETProSerSerTyrAspLeu

750 760 770 780 790 800
GGGTATCCTTACGCCGGAAGAAAAGAGCACGGTGATTTCCAAATTACTTGGAACTGTGA
GlyIleLeuThrProGlyArgLysGluHisGlyAspPheGlnIleThrTrpAsnCysGlu

810 820 830 840 850 860
AGGTAACGAGCCGATAAAAACAGCGCTAACAGCTAGCATTATCTCTGGCGTAGCAGAGGG
GlyAsnGluProIleLysThrAlaLeuThrAlaSerIleIleSerGlyValAlaGluGly

870 880 890 900 910 920
AGACAATAAGGTTTCGCCTGATTGCCAACAATCAGGCCTCAGGAGCAACTCTTTTTCTGAA
AspAsnLysValArgLeuIleAlaAsnAsnGlnAlaSerGlyAlaThrLeuPheLeuLys

930 940 950 960 970 980
GGAAAAGAATAGTGGTTCTCTGATAAACTGACTGGTCATGATGCACAGGATTATTTTGTG
GluLysAsnSerGlySerLeuIleLysLeuThrGlyHisAspAlaGlnAspTyrPheCys

990 1000 1010 1020 1030 1040
TAGTGACAGTCGTGAAACAACAGGCATGCGCATCTGTACGCTGATTCCGGTGACTGAGAC
SerAspSerArgGluThrThrGlyMETArgIleCysThrLeuIleProValThrGluThr

Fig. 6 - Continuation 1

11/18

1050 1060 1070 1080 1090 1100
TAGTTTGATAGGGCCGTTTGGTCGAGGGTATTCTACATTAAGTTTGTCTATTGCATATCC
SerLeuIleGlyProPheGlyArgGlyTyrSerThrLeuSerPheAlaIleAlaTyrPro

1110 1120 1130 1140 1150 1160
GTAAGTTGTAATATAAGCGGATTTTAATCTGTCTGGTGCTTATCGTTGCAACGAGCTATT

1170 1180 1190 1200 1210 1220
TTAGTAGCGGCTTCAGAAATGTTGTTTTCTGAAAGTCATTCTGATAAACAAACATAACAGT

1230 1240 1250 1260 1270 1280
TGTGCCGGATTTCTATTTTACAACCTGTGGAAGTAATTTGATGCAATGGAACTACTGGG

1290 1300 1310 1320 1330 1340
ATAATTCAAGTGTAACAGAGTCATCGAGTACCAAAGTATGATGTACGTTTGGCTGGTAA

1350 1360 1370 1380 1390 1400
AAAGCGAGTCGGATTAAGATCTTTATCTTAACACTTTATTCAATTTCTTGATTAACAAAAA

1410 1420 1430 1440 1450 1460
TATGCGTTTAAATATATCTTGATCAATCCGTTACTCTTGATTTCTTTGTCTGTGTTTGC
METArgLeuLysTyrIleLeuIleIleProLeuLeuLeuIleSerLeuSerValPheAla

1470 1480 1490 1500 1510 1520
GTCTACTCTACAAGTAGACAAGTCTGTTTCATACGACTGGATCAATTCTAGTGGGAGTAG
SerThrLeuGlnValAspLysSerValSerTyrAspTrpIleAsnSerSerAlaSerSer

1530 1540 1550 1560 1570 1580
TGCTCAAGTCACAGGAACACTTCTTGGCACAGGGAAGACAAACACTACCCAAATGCCAGC
AlaGlnValThrGlyThrLeuLeuGlyThrGlyLysThrAsnThrThrGlnMETProAla

1590 1600 1610 1620 1630 1640
TCTGTATACGTGGCAGCATCAAATCTACAATGTTAATTTCAATTCCTAGTTCATCAGGAAC
LeuTyrThrTrpGlnHisGlnIleTyrAsnValAsnPheIleProSerSerSerGlyThr

1650 1660 1670 1680 1690 1700
TTTGACATGCCAGGCTGGAACATTTTGGTATGGAAAAATGGGCGCGAAACCCAATATGC
LeuThrCysGlnAlaGlyThrIleLeuValTrpLysAsnGlyArgGluThrGlnTyrAla

1710 1720 1730 1740 1750 1760
GCTCGAGTGTCTGTGAGCATTACCATAGTTCTGGCTCCATTAATGAATCTCAGTGGGG
LeuGluCysArgValSerIleHisHisSerSerGlySerIleAsnGluSerGlnTrpGly

Fig. 6 - Continuation 2 12/18

1770 1780 1790 1800 1810 1820
GCAACAGTCACAAGTAGGATTCCGGTACAGCATGTGGAAATAAAAAATGTCGATTTACTGG
GlnGlnSerGlnValGlyPheGlyThrAlaCysGlyAsnLysLysCysArgPheThrGly

1830 1840 1850 1860 1870 1880
TTTGTAGATCTCTTTACGTATTCTCCTAATGCTCAGACATATCCCCTTTCTTCCGGTGA
PheGluIleSerLeuArgIleProProAsnAlaGlnThrTyrProLeuSerSerGlyAsp

1890 1900 1910 1920 1930 1940
TCTAAAAGGAAGTTTTCTTAAACCAACAAGGAGGTCAACTGGTCTGCTTCAATCTATGT
LeuLysGlySerPheSerLeuThrAsnLysGluValAsnTrpSerAlaSerIleTyrVal

1950 1960 1970 1980 1990 2000
TCCTGCGATTGCAAATCTTATAGACTAGACACGTCAATAACACTACCAGATACCGTGAA
ProAlaIleAlaLysSerTyrArgLeuAspThrSerIleThrLeuProAspThrValAsn

2010 2020 2030 2040 2050 2060
TTTAAAACAAAACCAAGCTACGCCTGTTTATTATACATTTTCAGCACCTAGCGTTTCAA
LeuLysGlnAsnGlnAlaThrProValTyrTyrThrPheSerAlaProSerValSerAsn

2070 2080 2090 2100 2110 2120
TACTATTACAGGGATCCCAACCAAAATGATATGCCTTCATCTAATGTCAAACTTACCAT
ThrIleThrGlyIleProAsnGlnAsnAspMETProSerSerAsnValLysLeuThrIle

2130 2140 2150 2160 2170 2180
AGGGCCGATCCAATCCAAAACAAGTCTCTTTATGTGCAGCCCGATGCAACAGGCTCGTG
GlyProIleGlnSerLysThrThrAlaLeuTyrValGlnProAspAlaThrGlySerTrp

2190 2200 2210 2220 2230 2240
GTATGATTTGTCTAAGAGCATAACAATGACAGTAGTAAAATCCAGTATGCTCAACTTCAA
TyrAspLeuSerLysSerIleThrMETThrValValLysSerSerMETLeuAsnPheLys

2250 2260 2270 2280 2290 2300
AGGAATTAACGCAAAACCAGGCAATGTTACATTGTGCGGTGCCCATTTGTTTTCGAGATACA
GlyIleAsnAlaLysProGlyAsnValThrLeuSerValProIleValPheGluIleGln

2310 2320 2330 2340 2350 2360
GTAATTCTAAAATAAAATTACATCGCGTAATAAGTAGACCACATACAGTTGCAGGATGTA

2370 2380 2390 2400 2410 2420
CAAAAGTTGGTAACGCAGGTGTTTCATTAGTTGTAATTTGCGCCGACATCTACGGGAAATG

Fig 6. - Continuation 3 13/18

2430 2440 2450 2460 2470 2480
TTGGATGCGCTCTCGAGCCCGTAGACAAATCTTCCCTATAGTTTGAGCATCGAAGGGTTG

2490 2500 2510 2520 2530 2540
TCTATGGGCACGCCACATTTTCCCCCTGAATTTAAGGAAGGGGCTGTTCCGCAAGTAACT

2550 2560 2570 2580 2590 2600
GTAGCGGGGCTATTCTATCGCAGAAGTATCTGAGCTCTCGGCGCACGGCCTTTATAAATC

2610 2620 2630 2640 2650 2660
ACTTCGAGCTGTTAAGCCCGATAGTTCTGGACTGCTGGTACCAGATTTACTATCTGTCAG

2670 2680 2690 2700 2710 2720
AACAGATATTTCTCAGACTGAAAGCACAACTTAGACGTACTGAGGAAGAACGGGATATTC

2730 2740 2750 2760 2770 2780
CGAAGATGAGTGCAGGTATTTGAAGGAAGCTCGACTGAAGTGTGCTTTATCAACGAT

2790 2800 2810
CCTCTAGAGTCGACCTGCAGGCATGCAAGCTT

1 Gln Gln Gly Asp Val Lys Phe Phe Gly Asn Val Ser Ala Thr Thr Cys Asn Leu Thr Pro Gln Ile Ser Gly
 CAG CAA GGG GAT GTT AAA TTC TTT GGT AAC GTA TCA GCA ACT ACC TGT AAT TTG ACA CCA ATA AGT GGC
 107/86
 2S221/90
 S1274/88
 S674/72

20
 Thr Val Gly Asp Thr Ile Gln Leu Gly Thr Val Ala Pro Ser Gly Thr Gly Ser Glu Ile Pro Phe Ala Leu
 ACT GTA GGA GAT ACC ATT CAG CTT GGT ACT GTT GCA CCA AGC GGA ACT GGT AGT GAA ATT CCT TTT GCA CTG
 40
 Ala Arg
 107/86
 S221/90
 S1274/88
 S674/72

30
 Lys Ala Ser Ser Asn Val Gly Gly Cys Ala Ser Thr Lys Thr Ala Asp Ile Thr Trp Ser Gly Gln
 AAG GCT TCT TCA AAT GTT GGC GGT TGT TCC TTG TCC ACT AAA ACA GCT GAT ATA ACT TGG AGC GGC CAG
 50
 60
 70
 107/86
 S221/90
 S1274/88
 S674/72

Figure 7A

15 / 18

80 90
 Leu Thr Glu Lys Gly Phe Ala Asn Gln Gly Gly Val Ala Asn Asp Ser Tyr Val Ala Leu Lys Thr Val Asn
 TTA ACC GAA AAA GGT TTT GCT AAT CAA GGG GGG GTG GCA AAT GAT TCA TAT GTC GCT CTG AAA ACC GTG AAC

107/86
 S221/90
 S1274/88
 S674/72

??

100 110 120
 Gly Lys Thr Gln Gly Gln Glu Val Lys Ala Ser Asn Ser Thr Val Ser Phe Asp Ala Ser Lys Ala Thr Thr
 GGT AAA ACA CAG CAG GGG CAG GAG GTT AAG GCG TCG AAT AGC ACT GTA AGT TTC GAT GCA TCA AAA GCA ACT ACG

107/86
 S221/90
 S1274/88
 S674/72

130 140
 Glu Gly Phe Lys Phe Thr Ala Gln Leu Lys Gly Gly Gln Thr Pro Gly Asp Phe Gln Gly Ala Ala Tyr
 GAA GGT TTC AAA TTT ACT GCT CAA CTG AAA GGT GGT CAA ACC CCG GGT GAC TTC CAG GCG GCA GCG GCT TAC

107/86
 S221/90
 S1274/88
 S674/72

149
 Ala Val Thr Tyr Lys
 GCG GTT ACT TAC AAG

107/86
 S221/90
 S1274/88
 S674/72

Figure 7A (continued)

18/18

130 Thr Glu Gly Phe Lys Phe Thr Ala Gln Leu Lys Gly Gly Gln Thr Pro Gly Asp Phe Gln Gly Ala Ala Ala
 ACG GAA GGT TTC AAA TTT ACT GCT CAA CTG AAA GGT GGT CAA ACC CCG GGT GAC TTC CAG GGG GCA GCG GCT

140

149

107/86
 2134
 2171
 2173
 2203
 8199
 8813

149 Tyr Ala Val Thr Tyr Lys
 TAC GCG GGT ACT TAC AAG

107/86
 2134
 2171
 2173
 2203
 8199
 8813

Figure 7B (continued 2)

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 93/03390

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/31 C07K13/00 C07K15/28 A61K39/108 C12Q1/10
C12Q1/68 C12N1/20 C12N15/62 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K A61K C12N C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>INFECTION AND IMMUNITY vol. 60, no. 5, May 1992, WASHINGTON US pages 1963 - 1971 HEIN IMBERECHTS ET AL. 'Characterization of F107 fimbriae of Escherichia coli 107/86, which causes edema disease in pigs, and nucleotide sequence of the F107 major fimbrial subunit gene, fedA.' cited in the application see abstract see page 1963, right column, paragraph 3 see page 1965, right column, paragraph 4 - page 1966, right column, paragraph 1 see page 1966, right column, paragraph 3 - page 1967, left column, paragraph 1 see page 1968, right column, paragraph 1 - page 1969, right column, paragraph 1 --- -/-</p>	1-10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

11 April 1994

Date of mailing of the international search report

04. 05. 94

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Authorized officer

Montero Lopez, B

INTERNATIONAL SEARCH REPORT

Inter. nat. Application No

PCT/EP 93/03390

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 314 224 (AKZO, N.V.) 3 May 1989 see page 2, line 22 - line 25 see page 5, line 21 - line 36 -----	1, 16, 18, 19

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. Appl. Application No

PCT/EP 93/03390

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		DE-A- 3871489	02-07-92
		ES-T- 2042718	16-12-93
		JP-A- 1149734	12-06-89
		US-A- 5208024	04-05-93
